THE ANALYST

PROCEEDINGS OF THE SOCIETY FOR ANALYTICAL CHEMISTRY

THE SOCIETY OF CHEMICAL INDUSTRY

On Tuesday, July 10th, 1956, in the theatre of the Royal Institution, London, the President, Dr. K. A. Williams, and the Honorary Treasurer, Dr. J. H. Hamence, presented the following Address to the Society of Chemical Industry on the occasion of the celebration of the 75th Anniversary of its foundation—

AN ADDRESS TO THE SOCIETY OF CHEMICAL INDUSTRY

On the occasion of the Celebration on July 10th, 1956, of the Seventy-Fifth Anniversary of the Foundation of the Society of Chemical Industry, The President, Officers, Council and Members of The Society for Analytical Chemistry send Greetings to the Officers and Members of the Society of Chemical Industry.

The Society sincerely welcomes this opportunity to express its appreciation of the goodwill and friendship that has existed between the Societies for the past three-quarters of a century, pays tribute to the past achievements of the Society of Chemical Industry, which has so signally advanced the study of all matters connected with Applied Chemistry, and offers good wishes for its continued prosperity.

Signed and Sealed on behalf of

The Society for Analytical Chemistry.

(Signed) K. A. Williams (*President*).
J. Hubert Hamence (*Honorary Treasurer*).
Noel L. Allport (*Honorary Secretary*).

Seal of The Society for Analytical Chemistry

Modern Qualitative Analysis and Industrial Practice

By C. J. van NIEUWENBURG

(*Lecture delivered at the meeting of the Midlands Section on Tuesday, March 6th*, 1956)

When speaking about "qualitative analysis," it might be well first of all for me to call attention to the fact that this expression decidedly has a double meaning. On the one side, it is hardly necessary to say that it is the branch of chemical science that the full-grown chemist applies when he wishes to know what elements or groups or compounds are present in a substance under investigation. It is perhaps wise to include in the definition that moreover he would like to know what are the major and minor constituents and what constituents are only present in traces. Anyhow, it belongs to the activities of finished chemists, engaged in serious chemical research. But there is quite another qualitative analysis, a set or scheme of operations intended to teach the elements of laboratory technique to the very youngest groups of chemistry students in our universities. As often as not, this is something quite apart, not aiming in the first place at true and completely reliable results, but rather at a didactical end.

In a great many chemical circles this ambiguity has caused a very regrettable contempt for qualitative analysis, which one meets even now, although the tempestuous growth of qualitative analysis in these last decades has considerably altered and improved the situation. I hope that this lecture may make a modest contribution towards removing the last remainders of this contempt by showing you that by now qualitative analysis has indeed come into its own.

Some thirty years ago it looked as if no branch of chemical science was as dead as qualitative analysis, but as you will know it rose from its ashes like a phoenix, being now full of new life. So, in order to understand what is essential in modern analysis, I should like to give you a short survey of its historical growth.

Like gravimetric analysis, qualitative analysis was born about the middle of the sixteenth century from the needs of metallurgy. About that time Agricola first studied the colours that different mineralogical products imparted to a colourless flame. However, the true father of this branch of analysis was your fellow-countryman Robert Boyle, who about the middle of the seventeenth century introduced the first veritable "reagents," substances specially intended to show the presence of some element. Of course that was only possible after he had given the first serviceable definition of an element, indeed based on its analytical recognisability throughout a number of compounds. From then on the notions of elements and qualitative analysis were indissolubly connected. Moreover, it is interesting to note that a number of Boyle's reagents were natural organic dyes, just as in modern analysis. The next essential improvement was due to the Swede Torbern Olaf Bergman, who towards the end of the eighteenth century laid down the principles for separating the then known metals into groups by means of hydrogen sulphide and sodium sulphide. This was the first "systematic" qualitative analysis. The idea has been elaborated by Berzelius and by the German school of nineteenth-century analysts like Klaproth, Rose and Fresenius, and given a more or less definite shape by the Swiss Treadwell. Together they built up the well known system of classical qualitative macro-analysis, which has since then practically remained unchanged. It is this "fixed" system which gave rise to the presumption that as a branch of science qualitative analysis was definitely dead. Let us admit that it was profoundly asleep during the first quarter of the present century; but I regret to say that there are still a great many universities where the preparatory teaching of it never passed this stage.

However, the germ of rejuvenation was already present. As long ago as 1679 my fellow-countryman and, in fact, fellow-citizen, Anthonie van Leeuwenhoek, the founder of microbiology, presented a paper to the young Royal Society of London, entitled "On the Figures of Salts," in which he showed that it is possible to identify chemical substances by means of their crystal shape under the microscope, the root idea of qualitative microscopic analysis. In the first half of the nineteenth century this principle was worked out by two naturalists, the Frenchman Raspail and the Dutchman Harting, and somewhat later by

Bořicky of Prague and by Haushofer of Munich University. It came to full growth about 1890 by the studies of a third fellow-countryman of mine, and again a fellow-citizen, Behrens, who gave us a complete list of reactions and reagents for all the more common elements. I won't treat of the modern development of microscopic analysis. Suffice it to say that in the United States important contributions were made by Chamot and Mason, and in the Argentine by Martini.

I was privileged to work from the very beginning of my chemical studies, that is now nearly fifty years ago, in the laboratories of Delft Technical University, where the Behrens technique was assiduously cultivated, and I learnt to appreciate its great merits. Of course it opened up quite a new field of analytical activity, the complete qualitative analysis of minute quantities of a substance, far below one milligram. In a great many cases this is extremely useful, and yet, in my opinion, that is not its greatest merit. I think it far more important that, as a rule, the microscopic reactions admit of a degree of certainty which is hardly ever attained by the old technique. They give us a set of excellent identity reactions, which can hardly be surpassed.

Under its influence the general character of qualitative analysis changed. More and more it came to consist of a limited number of separations, and, superimposed on these and inseparably connected with them, a set of identity reactions for the different elements within a certain group.

In due time the question arose whether these microscopic reactions were the only possible set of identity reactions, and of course they were not. It is the great merit of Feigl, formerly of Vienna University, now of Rio de Janeiro, to have opened up, if not invented, the large field of what is now known as drop-reactions or spot-tests. Since Feigl started work about 1925, a very great number of organic reagents have been found to give colour reactions of such a striking nature that all doubt whether a certain element is present, or not, is removed. In many respects these drop-reactions, like the microscopic reactions, provided us with an excellent set of new identity reactions.

Indeed, what we really were in need of some twenty years ago was a set of identification reactions of such a glaring and convincing nature that all doubts would be removed. This feeling of uncertainty was the greatest drawback of the old qualitative systems of Fresenius and Treadwell. They said that when at such and such a moment of the systematic course you obtained a white precipitate, then it was aluminium hydroxyde, or barium sulphate. I admit that indeed, if everything went accurately according to schedule, it was. But we now know that because of all sorts of co-precipitations and other complications, things are not so simple as the textbooks of that time told us, and the white precipitate might just as well have been some remnant of a preceding group. Generally speaking, I think that one may say that this feeling of uncertainty is by now quite out of date. The modern procedures, using the old trusted principles of separation into groups, or rather the critically mistrusted separations, but now with a set of fully reliable microscopic and drop-reactions superimposed on them, leave no more room for any reasonable doubt.

It seems futile to me to ask which of the two is the better set. Both have their own advantages and drawbacks, and I would rather say that they are complementary to one another. As a rule, drop-reactions are easier to carry out than microscopic reactions and require less experience, but, apart from some exceptions, they are definitely less selective. A drop-reaction is hardly ever really specific, whereas a great many microscopic reactions are, especially when combined with careful measurements of details like crystallographic and extinction angles and birefringence. But, of course, this requires proper apparatus, whereas the technique of drop-reactions can be carried out by very simple and cheap means. Perhaps this explains why up to now in many countries drop-reactions have been taken into the regular courses for training young students in qualitative analysis, and the microscopic technique, on the contrary, is restricted to a very limited number of university institutes. This is a pity. In analytical chemistry—in teaching it as well as in its practical application —we must be eclectic and not dogmatic or even fanatic. We must know and be master of as many different techniques as possible, and then, in each separate case, choose the one which seems to be the best suited. I won't deny that I fell into the same trap. Nearly forty years ago in my own institute I started with microscopic reactions as the only set of identity reactions. Then in 1927 I had the occasion to acquaint myself with the drop-reaction technique under the personal guidance of Feigl in Vienna, and I became so enthusiastic that I completely switched over to it; but that was a mistake. For a number of years now

we have practised qualitative analysis, even with very young students, in such a way that, as a rule, a microscopic reaction as well as a spot-test is carried out on each element, and this works to our complete satisfaction.

The same idea has been followed in the Reports of the Committee on New Reagents of the International Union of Chemistry. As long ago as 1934 it was evident that the number of identity reactions had become so large that it was hardly possible any longer for an inexperienced worker to find his way through the labyrinth. The Union formed a small committee to separate the chaff from the wheat and to collect a very restricted number of really recommendable identity reactions. I had the honour to act as its president from 1934 until 1949, and it is now under the guidance of Professor Gillis of Ghent University in Belgium. In its Second Report in 1945 the Committee discharged the greater part of its duty by giving the requested selections, whenever possible one or more drop-reactions and some microscopic reactions.

It is interesting to note that for nearly a hundred years people have tried to abolish hydrogen sulphide from qualitative analysis and that nevertheless it is still going strong. Apart from its disagreeable smell, it has the drawback of being a gas, with all the complications that arise from that. It can be used as an aqueous solution—and is indeed largely used as such in Germany—but this solution is not stable in contact with the air. Among the principles proposed to avoid using it, there are three worth mentioning. In the first place there is the use of mixtures containing sodium sulphide as advocated by Vortmann. This works quite well, but has the disadvantage of wasting tremendous amounts of chemicals, and of producing hydrogen sulphide at the most unexpected moments, which makes the remedy worse than the evil. Then there have been a number of methods in which zinc, cadmium or aluminium was used to reduce the solutions to metallic precipitates. As far as my experience goes, they all give rise to incomplete separations. And finally in these latter years the use of thioacetamide is largely advocated. I won't deny that it is possible to obtain reasonably good results with it, but that is not so easy, and moreover I think that in the long run the sweetish smell of thioacetamide is even more annoying than that of hydrogen sulphide. Personally I think that hydrogen sulphide is still best and I know by experience that it is quite possible to use it without producing any inconvenient smell in the working room.

Another problem worth discussing is whether it is really necessary to stick to the old separations. To my way of thinking the answer depends on what we are aiming at. For the time being I should not like to part with them in student courses. Here the teacher knows quite well what is in the mixture he gives out, and the only purpose of the whole course is to teach the students chemistry in general, and very simple analysis in particular. For them the separations are of too great a didactical value to throw them overboard, even if that should be possible. But for a research worker the situation is quite different. He aims at nothing but finding the constituents in as short a time as possible, always provided that this expedition does not impair the complete reliability of the results. It is evident that if the work could be done without separations, by simply consecutively applying a series of spot-tests or microscopic reactions, he would be fully justified in doing so. Now such collections of tests have indeed been proposed, for the cations by Charlot and Bézier of Paris by means of spot-tests and by Steimetz of Nancy by means of microscopic reactions, and for the anions by Tananaev of Kiev. One couldn't deny that they have been set up with remarkable ingenuity and skill and a profound knowledge of the subject. And yet I am rather afraid of such systems. The influence of other ions on redox potentials and on complex formation in unknown mixtures is so incalculable that I don't quite see how it is possible always to foresee all contingencies. Anyhow it seems to me that for the time being extreme caution must be recommended.

With all these new identification reactions, both microscopic reactions and spot-tests, which are equally well carried out on the micro or semi-micro scale, and no less because of the complete change in the apparatus used, drop-plates instead of test-tubes, centrifuges instead of funnels and filter-paper, and so on and so on, qualitative analysis has now been established on a micro-scale. To my way of thinking the change from macro to micro is here no longer a problem worthy of serious discussion. In qualitative analysis the micro-technique is in all respects preferable, and this statement holds good for actual research practice just as well as for preparatory university courses. It does the trick better and considerably faster, and moreover—very important for teaching purposes—more elegantly. Perhaps you may think that that is putting it rather strongly, but indeed I feel quite strongly

on this point. In the past, teaching of qualitative analysis has been one of the sloppiest and shoddiest parts of experimental chemistry tuition. As often as not it was as if teachers said: let us turn the young students loose in their first term on bottles and test-tubes and stinks, to their heart's content. They will automatically take a dislike to it, and tidiness and cleanliness will come later on. Maybe sometimes the trick worked, but I think that a great many students were spoiled for ever. Elegance and acquiring a good style are of paramount importance in all scientific work, but more especially during the first years of university tuition.

In my Institute we are now giving out 20 mg of substance, amply sufficient for a complete qualitative analysis—10 mg for the cations and 10 mg for the anions—and I can only say that the results are completely satisfactory, far better than thirty years ago with the old technique, and that the room in which it is carried out, and the furniture in it, is more like a drawing-room than like an old laboratory room.

Speaking of didactical questions, it may be worth while to remind you of the curious fact that qualitative analysis, both in its classical and in its modern form, is one of the few branches of chemistry which up to now is practically without any sound theoretical foundation. Why is mercuric sulphide so much less soluble in water than, say, manganous sulphide? Why are barium sulphate and silver chloride so insoluble and magnesium sulphate and mercuric chloride, both such nearly related compounds, not? Why do copper salts give an intensive colour with, say, dithio-oxamide, and lead salts do not? Up to now these questions are practically unanswerable, so much so that one might say that finding new colour reactions is greatly a matter of luck, at least at the beginning. As a rule, one accidentally finds a colour of some ion with a certain organic compound, and then starts investigating related compounds and substituted derivatives until the best one has been spotted. But even then a great deal of luck comes in. It would be highly desirable and beneficial to the development of qualitative analysis if some day a clever theoretical chemist would give us a clue to guide us in our research.

The remarkable evolution of qualitative analysis in these latter years has considerably increased its importance and usefulness for industrial research. It has enabled industry to get a far better insight into the composition of the materials with which it is working, especially with regard to traces and with regard to the presence of the less common elements.

Don't let us forget that in a great many industries traces of some elements are quite essential, either for the success or for the failure of the process involved. Let me only remind you of modern metallurgy and of those industries which are based on fermentation processes, where as often as not the whole course of the process is determined by the presence or absence of trace metals. In the laboratories of these industries and in those of research on foodstuffs for man and beast, modern trace analysis has been developed, which in an amazingly short time has become of tremendous industrial, agricultural and hygienic importance. Now you will perhaps object that I am trespassing. I have been asked to speak of qualitative analysis, and trace analysis is largely a matter of colorimetric quantitative analysis. Of course, in a certain sense you would be right, but on the other hand it is well to remember that by far the greater part of all these modern colorimetric methods has grown out of newly found qualitative colour reactions. Indeed, one might say that one of the first aims of the modern trend in qualitative research is to foster colorimetry, which implies that the sharp boundary-line between qualitative and quantitative analysis is gradually disappearing.

It is hardly necessary to point out the importance of trace analysis in a great many existing industries, but I should like to draw your attention to the fact that quite probably it will become of even greater and more essential importance in the near future for all applications of atomic energy. Here, even more than in biological processes, minute traces of foreign elements can have a detrimental effect. Let me remind you of the effect of traces of boron in the graphite of the atomic piles, boron being clearly noxious even when present in less than one part in a million, and of the trouble caused by traces of some metals in the steel walling of the piles, which give rise to a secondary radiation. Radioactive isotopes are supposed to be made *in* the pile and not *on* its outer surface!

Another instance where industrial practice directly profits by the recent development of qualitative analysis is connected with the less common elements. Up to a short time ago these less common elements were practically neglected—and even worse than that, they were intentionally hushed up. When two analysts met they said: let us not speak of titanium or tantalum, because if we do, we shall have to determine them. This policy has not only been detrimental to the picture we built up of the material world around us, but it has also

deprived industry of a great many chances. Now one of the trends of modern qualitative analysis has been to put the common and the less common elements on a more equal footing to the benefit of our well balanced conception of the material world and of industry. And in the future it will be even more so, because the fission products in an atomic pile are produced regardless of their geochemical frequency.

There is at least one point where up to now qualitative analysis decidedly falls short in its service to industry. We still have the detestable habit of first pulverising and irreparably messing up and destroying the structure of every sample which happens to fall into our hands. Isn't it about time that, at least in qualitative analysis, we paid more attention to the possibility of showing the presence of the elements *in situ*, or *in loco*, in the original sample itself? This type of "topographical analysis" is as yet only in its veriest infancy. Prints have been made from polished metal surfaces on a wet reagent paper in order to locate the impurities, but without much success. It seems to me that finding the right techniques for this sort of work would be highly beneficial to a great many branches of science, and particularly to industry.

In regard to the future development of qualitative analysis, there remain three points worth discussing. The first is whether *chromatography* will radically alter it or not. You will probably be well aware that there are a great many analysts who think it will. I do not share that opinion. I don't want to slander chromatography; indeed, I am fully aware of its great merits for the separation of organic substances, and I know quite well that even in the domain of inorganic separations it has booked and is continually booking new and striking successes, especially by means of paper-partition chromatography. But notwithstanding all that, I am not very optimistic about its systematic and general introduction into qualitative analysis. Of course it is an exceedingly useful tool. Indeed, we knew that, long before chromatography proper had been invented. Separation of the components of a drop of a mixed solution on a disc of filter-paper had been practised as long ago as the middle of the nineteenth century, and we now know incomparably more of it all. But, nevertheless, in my opinion, chromatography at present is at the most one of the valuable but very precarious and fickle resources of qualitative analysis, and fundamentally new things will have to be found before it can be expected to replace integral parts of the present technique reliably.

Far more serious is the competition of *spectrography*. One might even ask whether the flare-up of qualitative analysis in these latter years is not in reality more like the convulsions of a moribund. Is there any sense in going on when, according to a great many people, in a few years spectrographic analysis will make it all obsolete and superfluous? Here also I am rather optimistic.

Of course I know that even now one must admit that emission spectrography can perform nearly all the tricks of ordinary qualitative analysis of inorganic substances, and that infrared, visible-light and ultra-violet absorption spectrography are rapidly gaining ground in the domains of gas analysis and the analysis of organic groups. And we may be sure that they are only in their infancy. Undoubtedly spectrographic analysis has some striking advantages over purely chemical methods. In the first place it is far more expedient, especially for routine work. In this respect is it unsurpassable. In a few minutes it gives us a photograph of the spectrum, which, at least in principle, permits us to establish the complete qualitative composition of the substance under investigation and gives at least a good estimate of the quantities. Moreover, this photograph can be kept for a long time and remains a valuable document for later reference. This is not only very important in case of litigation, but it also enables us to look for the presence of other elements which did not interest us or which we didn't expect in the first place. And finally, at least for some elements like the alkali metals, the alkaline earths, indium, gallium and many more, spectrography is more sensitive than any of the now known identity reactions, to say nothing of quite a number of less common elements for which it is the only means of detection.

On the other hand, it has some unmistakable drawbacks. Of course it is very expensive and, even worse, it can only be successfully carried out in well equipped laboratories which have at their disposal a specialised and highly skilled laboratory staff. Generally speaking, I think we may aver that spectrographic analysis is now limited, and will be limited for some time to come, to routine work in specialised laboratories. This alone is sufficient reason to claim a lasting right of existence for the old trusted chemical methods. But there is more. Even when carried out in well equipped and expertly staffed laboratories, spectrographic

analysis cannot as yet always attain the same degree of certainty as chemical analysis in the more limited sense of the word. When working with unknown or uncommon products, mistakes remain quite possible. For the time being it wouldn't do to be without the purely chemical methods, if only as a standby to fall back on in cases of doubt—one might say, as its conscience. For those analytical chemists who are working on the development of qualitative chemical analysis it would be extremely dispiriting to know that they were fighting a losing battle. To me it seems abundantly clear that they are not. Prophesying has always been a dangerous job. Nobody can foretell what spectrography will bring us in the next twenty-five years. It is sure to improve and assuredly more and more big laboratories will be going to use it in daily practice, perhaps for ultimate analysis, but certainly for getting a first preliminary notion, just to show the way for a more definite analysis, if and when necessary; and in these big laboratories it will be largely a matter of organisation to ensure an efficient co-operation between spectrography and the other branches of analysis.

For the time being its price is prohibitive for small laboratories, but the same can be said of a great deal of the apparatus of modern chemical analysis. It seems to me that in the very near future this will inevitably lead either to a concentration of the smaller units or to co-operation in such a way that at joint expense they keep up some institute where the more expensive apparatus is available.

But anyhow, it doesn't yet look as if spectrography is going to monopolise qualitative analysis. In my opinion the question is not so much *whether* we ought to go on with studying the purely chemical methods, but rather *how* to do so, knowing that in some respects spectrography is decidedly superior.

So, for instance, I think that we had better not waste too much time on "systematic" courses that pretend to give us the complete analysis of a completely unknown substance. This may be useful and perhaps necessary for training young university students, but in industrial and research practice we do not meet with what Biltz once called "the remains of an exploded drug-store," where strontium and manganese and tartaric acid and fluorides are to be found together. We had better leave such things to spectrography. What we are much more in need of are more selective, if possible truly specific, reactions, and particularly selective methods for the isolation or extraction of the various ions from those with which they are commonly associated. If we are to compete successfully with spectrography and with chromatography, we shall have to invent a great many more rapid methods which are nevertheless fully reliable.

Let us not be afraid of chromatography and spectrography; we have to use them and accept them as serious and perhaps even dangerous competitors, but anyhow as a stimulus to further research. For the time being purely chemical analysis and spectrography and chromatography each have their own reasons for existence, and it is our duty as conscientious chemists to find a happy synthesis of the three.

In conclusion there is the final point I should like to raise in connection with future development of qualitative analysis. Up to now we have always tried to find organic reagents for showing the presence of inorganic ions; that means that practically always we aimed at inorganic analysis. One is inclined to ask whether it wouldn't be possible to do it the other way round. Couldn't we find a set of inorganic ions that give selective reactions with certain organic groups, in such a way that we can use them for elucidating the constitution of organic compounds? Of course we know that nickel is a selective reagent for α-dioximes, that copper shows the presence of α-hydroxycarboxylic acids, and when we skim the second volume of Feigl's "Spot Tests," we find a great many other examples. But all this is purely incidental. I fully realise the difficulties of the problem, if only because the behaviour of organic groups is so often dependent on the presence of other groups in the molecule, but nevertheless it seems to me that systematic studies in this direction might be worth while.

Altogether, qualitative chemical analysis is fully alive again, bristling with problems, both didactic and of a purely chemical nature.

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The Determination of Uranium by High-precision Spectrophotometry

By A. BACON and G. W. C. MILNER

A procedure is described for determining the uranium content of relatively pure samples of U_3O_8 and uranium metal with a precision (1 σ) of $\pm$ 0·04 per cent. The sample is dissolved in nitric acid and this solution is then converted to standard conditions of acidity by evaporating to fumes of sulphuric acid. After dilution with water to a standard volume, the absorbancy of the sample solution is measured at 430 mμ, the reference solution being of accurately known uranium content. The uranium content of the sample is then obtained either by referring the absorbancy difference to a calibration graph or by calculation with use of a factor derived from the calibration graph.

The main factors that influence the accuracy of the determination are discussed in detail.

The accuracy required from the analytical chemist can vary very widely, but in recent years there has been an increasing demand for analytical methods that would give an accuracy of better than 99·9 per cent. Since about 1949, spectrophotometry has been developed to such an extent that this technique is now making a valuable contribution in high-accuracy work. Moreover, in specific cases spectrophotometric methods have decided advantages over conventional procedures, since they are rapid and simple in operation and chemical separations can generally be avoided. These methods have resulted from the introduction of the differential system of measurement, which employs reference solutions of high absorbancy. Hiskey[1] was among the first to show that the accuracy of the spectrophotometric methods can be increased by using reference solutions of high absorbancy. It does not follow, however, that under these conditions maximum accuracy can be attained within the concentration range in which solutions obey the Beer - Lambert law. For solutions failing to obey this law, it is possible to determine experimentally the concentration range for maximum accuracy.

It was thought that the differential spectrophotometric technique should be of advantage in the determination of macro-quantities of uranium, especially in uranium-base materials. The volumetric method for uranium is subject to serious interference from other elements, as the reagents used to reduce the uranium to the quadrivalent state before titration with ceric sulphate generally reduce many other elements. Moreover, the conventional gravimetric method for uranium, involving precipitation as ammonium diuranate followed by ignition to U_3O_8, also leaves much to be desired. Many other elements are precipitated by ammonium hydroxide under the same conditions and so cause contamination of the final U_3O_8. It was considered that the absorptiometric technique would be free from many of the above objections and would result in some improvement in the determination of macro-amounts of uranium.

The soluble compounds of uranium have been the subject of extensive research in recent years, the absorption spectra being used to postulate the ionic species present in solution. Kaplan, Hildebrant and Ader[2] studied the absorption spectra of uranyl nitrate in both aqueous and ketone media. Spectra for uranium in hydrochloric and perchloric acid solutions have been reported by Sutton.[3]

Recently, Rabinowitch[4] carried out a comprehensive survey of the absorption spectra of uranyl compounds. Spectra are shown for free uranyl ions and also for hydrolysis products, for uranyl ions complexed with acid anions and for uranyl compounds in organic solvents. Some indication is also given of the absorption spectra for solutions with pH values greater than 5.

A study of the available information revealed that the absorption spectra were least affected by changes in the acid concentration when the acidity was high. Further, the most effective control of acidity can be attained by evaporating sample solutions to fumes of sulphuric acid, and investigations were therfore limited to the determination of uranium in sulphuric acid solutions. Uranyl sulphate solutions absorb light at two wavebands, from

275 to 325 mμ and from 400 to 450 mμ, the corresponding peak absorbancy indices (g per litre per cm) being 1·1 and 0·055, respectively. Unfortunately light of the first waveband is subject to serious interference from many elements, including iron, molybdenum, niobium, zirconium and so on. Many of these difficulties do not arise with the higher waveband and, since the applicability of this technique was just as important as the best accuracy, investigations were limited to the higher waveband. Moreover, since the ultimate accuracy attainable in a determination is dependent on the largest single error from any one operation of the procedure, extensive experiments were carried out to assess the degree of control needed for the attainment of maximum accuracy under these conditions.

System for obtaining absorbancy values

The system used in this work consisted in reversing the solutions in the two cells reserved for absorbancy determinations, setting the instrument on zero, first by the reference solution in cell 1 and, when the solutions are reversed, by the reference solution in cell 2. Two readings are obtained with this system and, when the same two cells are used, the sum of the readings is directly related to the absorbancy difference; for a complete mathematical treatment reference should be made to the report on which this paper is based.[5] Under standard solution conditions it is shown, for example, that—

$$R_2 + R_4 = (b_2 + b_1)(A_2 - A_1) = -(R_1 + R_3),$$

where b_1 = path length of reference cell, 1, in cm and b_2 = path length of second cell, 2, in cm. During the exchange of the solutions, it is imperative that the intrinsic absorbancy error of the two cells should not change. The difference between the two readings is a direct measure of the constancy attained during the reading of any pair of solutions. The reading difference is dependent on the absorbancy of the reference solution, even when the same solution is used in both cells. When a series of reference solutions is used, therefore, a reliable value for each should be obtained, a graph constructed and close agreement to these values ensured before taking readings on test solutions. The use of the same solution in each cell ensures that solution errors are constant and the expressions for the reading differences[5] under these conditions are—

$$R_1 - R_3 = (b_2 - b_1)(A_1 + A_2) - 2E_1, \text{ and}$$
$$R_2 - R_4 = (b_2 - b_1)(A_1 + A_2) + 2E_1,$$

where E_1 is the intrinsic absorbancy error of cell 2 with respect to cell 1 (positive).

Typical values with the same uranium solution in each cell for three separate solutions are given in Table III A (see p. 466) and from these the mean values for $(b_2 - b_1)$ and E_1 have been calculated. These results have been used to derive the calculated values for $R_1 - R_3$ and $R_2 - R_4$ shown in the calibration data given in Table III. Comparison of these values against those obtained experimentally shows the maximum experimental error to be of the order of $\pm$ 0·05 per cent.

Principle of determining maximum accuracy—

Let C_1 = the concentration of the reference solution,
C_2 = the concentration of the test solution,
A_1 = the theoretical absorbancy of the reference solution under standard conditions in a 1·0-cm cell, and
A_2 = the theoretical absorbancy of the test solution under standard conditions in a 1·0-cm cell.

The concentration error at any point on a spectrophotometric calibration graph is defined exactly as—

$$\epsilon_C = \epsilon_A \times \frac{\Delta C}{\Delta A} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

where ϵ_C is the concentration error, ϵ_A is the error in reading ΔA and $\frac{\Delta C}{\Delta A}$ is the calibration factor.

The fractional error at any concentration C_1 is, therefore—

$$f_C = \epsilon_A \times \frac{\Delta C}{\Delta A} \times \frac{1}{C_1} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (2)$$

Now maximum accuracy is attained when the fractional error is a minimum. It follows, therefore, that maximum accuracy is attained when the following expression is at a maximum—

$$\frac{\Delta A}{\epsilon_A} \times \frac{C_1}{\Delta C} \quad .. \quad .. \quad .. \quad .. \quad .. \quad (3)$$

Hiskey[1] has shown that for solutions obeying the Beer - Lambert law $\frac{\Delta A}{\epsilon_A}$ is at a maximum when ΔA is 0·4343.

Since $\frac{\Delta A}{\Delta C}$ is constant, the maximum accuracy is attained when C_1 is at a maximum and, further, the fraction $\frac{\Delta A}{\Delta C}$ is best determined by using a differential system of measurement and a value for ΔC such that ΔA is 0·4343. For solutions that do not obey the Beer - Lambert law, however, $\frac{\Delta A}{\Delta C}$ decreases when the solutions fail to comply to this law. It follows, therefore, that a plot of the function $\frac{\Delta A}{\epsilon_A} \times \frac{C_1}{\Delta C}$ against C_1 will show a maximum where the rate of change of $\frac{\Delta A}{\Delta C}$ is equal to that for C_1.

Theoretically $\frac{\Delta A}{\Delta C}$ should be determined by using small values for ΔC and ΔA. Hiskey[1] has shown, however, that the accuracy in determining ΔA decreases rapidly below a value of 0·20; hence ΔC should be chosen such that ΔA is approximately 0·20; and ϵ_A must be maintained constant, this being attained by using the same value for ΔC at various values for C_1, which results in the reading being obtained at the same place on the logarithmic scale of the instrument, when the differential system of measurement is used.

Although for solutions that do not obey the Beer - Lambert law ΔA changes when ΔC is constant, for small changes in ΔA, ϵ_A can be still considered constant.

When ΔA is measured differentially, then either a change in the meter sensitivity or in the slit width is necessary to re-balance the instrument when the concentration of the reference solution is changed. In order to maintain constant slit width and yet attain satisfactory meter sensitivity, the sensitivity control should be adjusted against a reference solution of high concentration and a slit width chosen to give satisfactory meter response. Moreover, conditions should be chosen such that the sensitivity control can be used to re-balance the instrument when the concentration of the reference solution is altered. The range of concentrations used for the reference solution is, therefore, limited and only values obtained on solutions of high concentration are shown in Fig. 1.

The values for curve A were obtained by adding the absorbancy differences obtained for each increment of 4 g of uranium per litre to the absorbancy obtained for a solution containing 30 g of uranium per litre. The values for any concentration on curve B were calculated from the absorbancy difference obtained by using a reference solution containing 30 g of uranium per litre and a second solution containing 34 g of uranium per litre. They are, therefore, the theoretical absorbancies that would be obtained at concentrations of uranium higher than 30 g per litre if the solutions continued to obey the Beer - Lambert law.

The deviation of curve A from curve B shows the experimental deviation of the solutions from the Beer - Lambert law. The values for curve C were calculated by using the experimental absorbancy difference obtained for each increment of 4 g of uranium per litre, use being made of the following expression—

$$\text{Relative accuracy} = (A_2 - A_1) \times \frac{(C_1 + C_2)}{8}$$

This is obtained from equation (3) by substituting $\frac{C_1 + C_2}{2}$ for C_1, $A_2 - A_1$ for ΔA and 4 for ΔC. It can be seen that curve C shows the concentrations at which the solutions fail to comply with the Beer - Lambert law far more clearly than curve A and that maximum accuracy is attained when the concentration of the reference solution is about 48 g of uranium per litre, considerably greater than the concentration at which the Beer - Lambert law fails.

The corresponding absorbancy for maximum accuracy is about 2·2. When the meter sensitivity is such, therefore, that the instrument can be read with certainty to ± 0·1 per cent. transmission, then for small differences in absorbancy the theoretical relative error is ± 0·017 per cent. It can be seen, however, that the slope of the accuracy curve over the range 40 to 60 g of uranium per litre is relatively small. Hence the concentration employed for the reference solution is not critical. The use of a reference solution of lower concentration than the optimum favours the use of narrower slit widths and 40 g of uranium per litre was chosen for experimental calibration purposes.

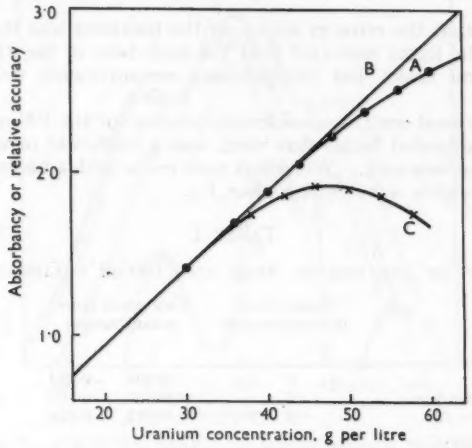

Fig. 1. Uranium concentration range for maximum accuracy with a Beckman spectrophotometer, 1-cm cell and 0·80-mm slit width (10·4 mμ): curve A, experimental absorbancy; curve B, theoretical absorbancy; curve C, relative accuracy

From the results obtained by using different slit widths, the generalisations that follow were deduced.

Decreasing the slit width results in an increase in the slope of the theoretical curve, A, the deviation of the experimental absorbancy from the theoretical decreases and the maximum accuracy, the optimum concentration for maximum accuracy and the concentration where solutions fail to comply with the Beer - Lambert law, all increase.

An approximate estimate of the extent of these changes is as follows—

Slit width, mm	*A*, g per litre per cm	Concentration at which Beer - Lambert law fails, g per litre	Optimum concentration, g per litre	Relative accuracy
0·4	0·048	40	54	2·1
0·8	0·047	32	48	1·9

Solution variables

From a mathematical consideration[5] it can be shown that, when solution conditions are identical for each member of a pair of solutions but different from standard conditions, then the individual points of the calibration graph and subsequent measurements referred to this graph will only be affected by the fractional error in each pair of solutions. When, however, solution conditions vary in each member of a pair, the validity of the results is influenced by both the fractional and intrinsic errors of each solution. Therefore, when a reference solution is prepared in bulk and retained as a permanent reference solution, great care should be taken to ensure that any subsequent solutions are prepared from exactly the same reagent solutions. The inclusion of a "control" reference solution amongst each batch of solutions is to be recommended as a check on the validity of the permanent reference solution. Moreover, the inclusion of a second "control" solution, different in concentration,

permits any errors to be classified as intrinsic or fractional and correction factors to be calculated.

The type and size of some of the errors introduced by variations in solution conditions are discussed in detail under appropriate headings.

CONTROL OF URANIUM CONCENTRATIONS—

Experiments were conducted to determine the precision that could be attained when 50 ml and 100-ml calibrated flasks are used for volume adjustment, and the results are shown in Table I.

The values show that the error in adjusting the meniscus was the same for both flasks. Casual inspection of the flasks indicated that the neck bore of the 100-ml flasks was larger than that of the 50-ml flasks, but internal-bore measurements revealed that they were the same.

Although the fractional error is considerably smaller for the 100-ml calibrated flasks, the four selected 50-ml calibrated flasks were used, and a coefficient of variation of $\pm$ 0·02 per cent. was accepted as satisfactory. Weighings were made with a balance having a sensitivity of $\pm$ 0·1 mg and all weights were greater than 1 g.

TABLE I

COMPARISON OF THE USE OF 50-ml AND 100-ml CALIBRATED FLASKS

Test	Number of determinations	Deviation from mean (range)	Standard deviation, ml	Coefficient of variation, %
With 50-ml flasks at 20° C—				
The same flask	6	+0·009, −0·011	±0·007	±0·014
6 flasks selected at random ..	6	+0·046, −0·100	±0·051	±0·102
4 flasks selected from 12	4	+0·012, −0·010	±0·010	±0·020
With 100-ml flasks at 20° C—				
The same flask	6	+0·010, −0·009	±0·007	±0·007
6 flasks selected at random ..	6	+0·031, −0·039	±0·024	±0·024
4 flasks selected from 12	4	+0·010, −0·012	±0·008	±0·008

CONTROL OF THE ACIDITY—

In the study of the effect of acidity on the uranium absorption spectra, a series of solutions was prepared from analytical-reagent grade uranyl sulphate, $UO_2SO_4.3H_2O$. The uranium concentration was maintained constant at 0·75 g in 50 ml and sulphuric acid additions were made so that the acidity varied over the range 0·5 to 18 *M* Typical spectra are shown in Fig. 2, from which it can be seen that for solutions less than 9 *M* in sulphuric acid, large changes in the final acidity do not influence the character of the uranium spectrum appreciably. At acidities in the region of 18 *M*, however, the character of the absorption spectrum is completely changed. When the absorbancy values were plotted against acidity for different wavelengths in the waveband 400 to 450 mμ, an optimum acidity was found for each wavelength at which the absorbancy is least affected by changes in the acidity. Optimum acidities of 2 and 4 *M* were obtained, for example, for wavelengths of 410 and 430 mμ, respectively.

The behaviour at 430 mμ was examined in greater detail by using the differential system of measurement, the result being shown graphically in Fig. 3. It can be seen that small changes in acidity will produce the least error when both the reference and the test solution are 4 *M* with respect to sulphuric acid. Further, when the acidity of the test solution differs from that of a reference solution that is 4 *M* with respect to sulphuric acid, the resultant error is always negative, irrespective of whether the test solution is greater or less than 4 *M* with respect to sulphuric acid. Moreover, the size of the error is dependent upon the extent of the difference in acidity between the test and sample solution. Approximating over the curve in Fig. 3 in the acidity range 3·5 to 4·5 *M* with respect to sulphuric acid, it is found that a positive or negative change of 12 per cent. in acidity from 4 *M* results in a negative error of — 0·10 per cent. In consequence the acidity difference between the reference solution and the test solution should not vary by more than 2 per cent. if a reproducibility of $\pm$ 0·02 per cent. is to be attained. With the system of preparing a bulk standard reference solution to be used as required over a long period, the acidity of the stock sulphuric acid used to

prepare this standard and that used at a later date to prepare sample test solutions should not vary by more than 2 per cent.

Experiments were carried out to determine the variations in acidity that occur when the procedure of evaporating to fumes of sulphuric acid is used for the removal of other solvent

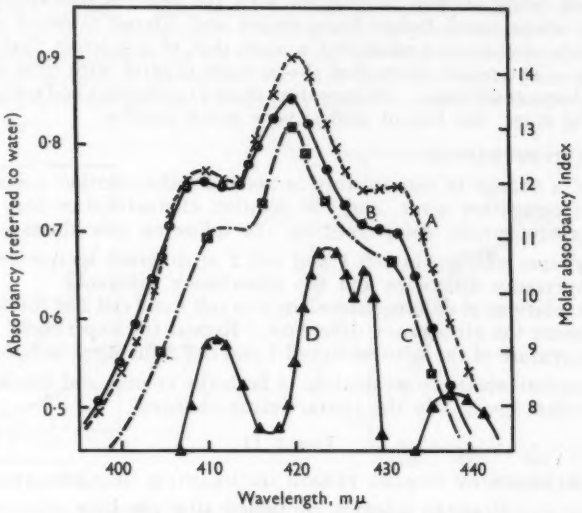

Fig. 2. Absorption spectra for uranyl sulphate measured on a Beckman spectrophotometer with 1-cm cell and 0·05-mm slit width (0·5 to 0·9 mμ), the uranium concentration being 15 g per litre (0·063 *M*): curve A, 3·0 *M* sulphuric acid; curve B, 0·5 *M* sulphuric acid; curve C, 9 *M* sulphuric acid; curve D, 18·0 *M* sulphuric acid

acids. Experimental results in the absence of uranium showed that standard acidity can be attained to about ± 2 per cent. by using a fuming period of 10 minutes ± 5 minutes. No difficulty was experienced in removing hydrochloric or perchloric acids. With nitric acid, however, diluting the solution with water after a single fuming resulted in the formation

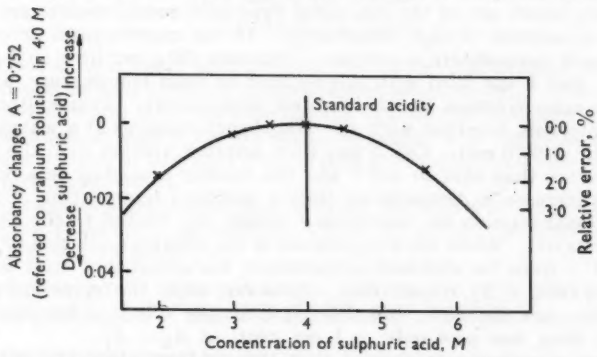

Fig. 3. Relative error for deviations from standard acidity with a Beckman spectrophotometer, 1-cm cell and 0·25-mm slit width (3·25 mμ), the uranium concentration being 15 g per litre and the temperature 23° C

of brown fumes, which indicated the presence of some residual nitrogen compounds. This behaviour is associated with the formation of nitrosylsulphuric acid, and the amount formed seemed to be dependent on the concentrations of the acids when mixed. Although no serious errors could be attributed to traces of residual nitric acid, double fuming was considered

desirable. Results obtained in the presence of uranium are given in Table II. In the first set of experiments, several 2·3585-g portions of selected U_3O_8 were taken and dissolved in minimum amounts of nitric acid, sp.gr. 1·42. An accurately measured 20-ml portion of 20 *N* sulphuric acid was then added to each beaker, and the solutions were evaporated to fumes, the process being allowed to proceed with the beakers uncovered. The solutions were fumed for various times, before being cooled and diluted to 50 ml with water. The absorbancy of each solution was measured against that of a solution that had been fumed for 1 minute only. The results show that the increase in error with time of fuming is quite significant under these conditions. On repeating these experiments and using covered beakers during the fuming stage, the loss of acid is very much smaller.

CONTROL OF THE TEMPERATURE—

The effect of a change in temperature is twofold[6]; the solution volumes are changed, resulting in a concentration error, and the solution characteristics may alter. The two effects can be experimentally determined by the following procedures—

(*i*) Use the same solution in cell 1 and cell 2 at different temperatures and measure the temperature difference and the absorbancy difference.

(*ii*) Use two solutions of differing absorbancy in cell 1 and cell 2 at the same temperature, and measure the absorbancy difference. Repeat the experiment, still maintaining the temperature of the solution in cell 1 and cell 2 the same but at a different value.

The first procedure gives the summation of both the volume and characteristic changes. The second procedure gives only the characteristic changes.

TABLE II

INFLUENCE OF FUMING PERIOD ON URANIUM DETERMINATION

Uranium solution contained 40 g per litre.

Fuming in an open beaker—				
Time of fuming, minutes	1	2	3	5
Error, %	0	−0·06	−0·18	−0·30
Fuming in a covered beaker—				
Time of fuming, minutes	2	5	10	30
Error, %	0	−0·06	+0·06	+0·09

The first system is simple in experimental operation. The volume change can be assessed from specific-gravity tables or specific-gravity determinations, the error calculated and the characteristic error derived.

Temperature errors are of the fractional type and measurements are best conducted, therefore, with a solution of high absorbancy. In the experimental determination of the effect of changes in temperature a solution of uranium (36 g per litre) in 4 *M* sulphuric acid was employed. Cell 1 was filled with this solution at room temperature, whereas cell 2 was filled with the same solution at an elevated temperature. Temperature measurements were taken at intervals, together with the absorbancy readings at a wavelength of 430 mμ, with a slit width of 0·70 mm. Cell 2 was then emptied, refilled with the same solution at a lower temperature than that in cell 1 and the reading procedure was repeated. Results showed that an increase in temperature from a standard temperature of 23° C resulted in a positive fractional error in the absorbancy, which was related to the deviation from the standard temperature. When the temperature of the reference solution or the test solution differed by ± 8° C from the standard temperature, the absorbancy error was ± 1 per cent. of A_2 or ± 1 per cent. of A_1, respectively. However, when the temperature of the reference and test solutions was the same, but differed from the standard temperature by ± 6° C, the absorbancy error was now only ± 1 per cent. of $A_2 - A_1$.

Temperature measurements showed that the gradient along the cell housing of the Beckman instrument could be as high as 1·5° C and that the temperature in the cell housing was dependent on how long the lamp had been switched on. Both the lamp housing and the cell carriage were, therefore, fitted with water jackets, a circulating pump was installed and the circulating water was thermostatically controlled at a standard temperature. Time was always allowed for the solutions to acquire the same temperature in the cell compartment.

A water bath was also thermostatically controlled so that solutions could be diluted to volume at the standard temperature.

Spectrophotometer variables

Relationship between slit width and meter sensitivity—

It is essential in precision spectrophotometry that the meter sensitivity should be so adjusted that a 0·1 per cent. change in transmission will give a detectable movement of the galvanometer needle. Also the instrument must be sufficiently stable to ensure that fluctuations are less than the above deflection. To maintain this meter sensitivity as the absorbancy of the reference solution increases the slit width must be increased to re-balance the instrument.

A plot of the minimum slit width that will give the above sensitivity against the absorbancy of the reference solution, for the two ranges available on the Beckman instrument, is given in Fig. 4.

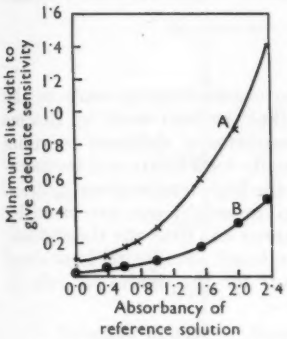

Fig. 4. Relationship between absorbancy and slit width to give adequate sensitivity with a Beckman spectrophotometer for uranium solutions in 1-cm cells at 430 mμ: curve A, sensitivity setting 1·0; curve B, sensitivity setting 0·1

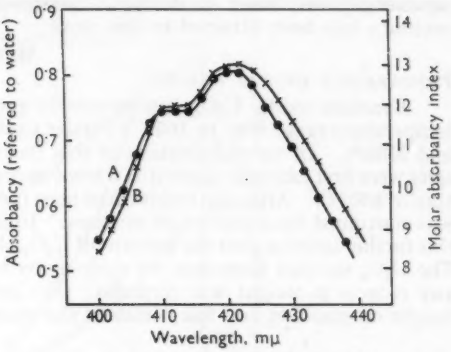

Fig. 5. Absorption spectra for uranyl sulphate with a Beckman spectrophotometer and 1-cm cell at an increased slit width of 0·40 mm (4 to 13 mμ), the uranium concentration being 15 g per litre: curve A, 0·5 *M* sulphuric acid; curve B, 3·0 *M* sulphuric acid

According to Hiskey,[7] the ratio of the slit widths for the reference solution and for the water blank is related to the intensities of the transmitted light by the expression—

$$\frac{I_w}{I_s} = \left(\frac{\text{slit width for solution}}{\text{slit width for water}}\right)^r,$$

where r is a function of light losses due to setting the mirrors and aligning the optical system. The value for r should be 2 for correctly adjusted spectrophotometers. When the absorbancy values obtained for uranium solutions were plotted against the logarithm of the ratio of the slit width for the solution against the slit width for water, a straight-line calibration graph was obtained with a slope of 2. This result verified that the Beckman spectrophotometer used was in correct alignment.

Further experiments showed that at a constant slit width the meter sensitivity varied inversely as the transmission of the solution. Thus slit width, transmission and sensitivity can be intercorrelated,[7] as follows—

$$\left(\frac{\text{slit width for water}}{\text{slit width for solution}}\right)^2 = \frac{\text{transmission for solution}}{\text{transmission for water}} = \frac{\text{meter sensitivity for solution}}{\text{meter sensitivity for water}}.$$

Wavelength and waveband selection—

With the normal light source of the Beckman instrument wide slit widths are necessary to obtain adequate meter sensitivity when reference solutions of high absorbancy are used. This arises because it is impracticable to increase the intensity of the light source. The effect of increasing the slit width on the plot of the absorption wavelength can be seen by comparing Fig. 5 with Fig. 2. By increasing the slit width eightfold, the irregularity in the absorbancy - wavelength plot at 430 mμ (shown in Fig. 2) has entirely disappeared, whereas

the one at 412 mμ has been hardly affected. Further, the peak height at 420 mμ has decreased considerably.

Doubling the slit width to 0·8 mm produced little change from the absorbancy values shown in Fig. 5, and it follows that the use of wide slit widths considerably decreases the error introduced by inaccurate setting of the slit width.

Errors can arise, however, from inaccurate setting of the wavelength scale and these are at a minimum when the smallest change in absorbancy occurs for unit error in setting the wavelength scale. From Fig. 5 the corresponding wavelengths that satisfy this requirement are 412 and 422 mμ. Unfortunately, interference by such elements as niobium and molybdenum (proposed alloying constituents for uranium-base alloys) is significant at these wavelengths. At 430 mμ, however, the above interference is less troublesome and this wavelength was chosen for detailed investigation. It is not claimed, therefore, that ultimate accuracy has been attained in this work.

Method

Preparation of the oxides—

Uranium oxide, U_3O_8, can be readily produced by igniting uranium compounds in the temperature range 800° to 1050° C, under oxidising conditions[8] (but see later work by Brouns and Mills[9]). In an examination of this technique weighed quantities of different uranium salts were first carefully ignited at a low temperature and then finally for 3 hours at a temperature of 850° C. After the residue had been cooled and weighed, the high-temperature ignition was continued for a further 30 minutes. In all cases, no change in weight was produced by this further ignition, but the amount of U_3O_8 obtained was sometimes less than the theoretical. The U_3O_8 samples were next set aside under normal atmosphere conditions for 60 hours and any change in weight was recorded. The general tendency was for the oxides to gain in weight on standing and full details of the results are as follows—

Uranium compound	Deviation of weight of U_3O_8 from weight theoretically expected, %	Gain in weight of U_3O_8 on exposure to atmosphere for 60 hours, %
$UO_2(C_2H_3O_2)_2.2H_2O$ (AnalaR)	−4·64	0·05
$UO_2SO_4.3H_2O$ (laboratory reagent)	−3·06	0·02
$UO_2(NO_3)_2.6H_2O$ (AnalaR)	nil	0·03
UO_3 (laboratory prepared)	−3·09	0·012

Further evidence in support of the results in the last column was obtained from the examination of Specpure U_3O_8 and a specimen of high-purity U_3O_8 prepared by chromatography on cellulose. The full history of these samples was unknown, but the first had a loss in weight of 0·27 per cent. on ignition and the second a loss of 0·71 per cent. All this evidence emphasises the necessity of igniting all U_3O_8 samples to constant weight before use.

Solutions required—

Sulphuric acid, 20 N, standard stock solution—Cautiously pour 555 ml of AnalaR sulphuric acid, sp.gr. 1·84, into 400 ml of water, while cooling the solution. Dilute to 1 litre at room temperature with water. When a further stock solution is prepared, it should be within 2 per cent of the standard stock solution.

Standard uranium solution (primary reference solution)—2·3585 g of the selected U_3O_8 previously ignited to constant weight and stored in a desiccator were weighed for every 50 ml of solution required, transferred to a tall lipped conical flask and dissolved in the minimum amount of nitric acid, sp.gr. 1·42, and 20 ml of the stock sulphuric acid solution were added (from a burette to ± 0·1 ml) for every 50 ml of solution required. The solution was boiled down and fumed 10 minutes, cooled, diluted to about four times the volume, evaporated and fumed for a further 5 minutes.

The solution was cooled and diluted to the appropriate volume at a standard temperature of 23° C. The 250-ml calibrated flask was standardised against the 50-ml flasks subsequently used for preparing other solutions, and the appropriate weight of U_3O_8 required was calculated by using the following equation—

$$\text{Weight of } U_3O_8 = 2{\cdot}3585 \times y/x,$$

where x = weight of water contained in standard 50-ml flask at 23° C, and

y = weight of water contained in the 250-ml flask at 23° C.

Reference solution—2·3585 g of U_3O_8 were processed as previously described. The solution was diluted to volume in a 50-ml calibrated flask at 23° C.

Test solution—Various weights of U_3O_8 were processed as previously described and the solutions were diluted to volume in selected 50-ml calibrated flasks at 23° C.

Sample solution—2·3585 g of the U_3O_8 samples (2 g of the uranium metal) were processed as previously described and the solutions were diluted to volume in selected 50-ml calibrated flasks at 23° C.

PROCEDURE FOR CALIBRATION—

The accuracy of the calibration graph at any concentration is dependent on the absorbancy difference used to determine its slope. For solutions that obey the Beer - Lambert law the slope can be determined with maximum accuracy when the absorbancy

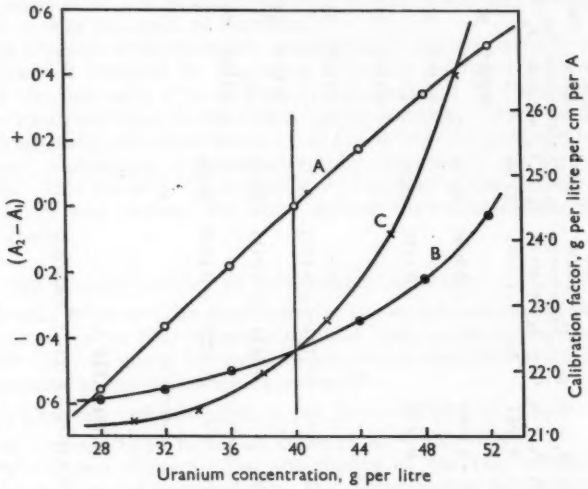

Fig. 6. Calibration graph for uranium by using 1-cm cells, 0·8 mm slit width (10·4 mμ) at 430 mμ and a temperature of 23° C: curve A, absorbancy differences versus the uranium concentration (reference solution containing 40 g of uranium per litre); curve B, calibration factor versus the uranium concentration (reference solution containing 40 g of uranium per litre); curve C, calibration factor versus the average uranium concentration (multiple reference solutions)

difference between any two solutions is 0·4343. The concentration range covered by one reference solution is dependent on the accuracy required over that range, and the choice of the concentration for the reference solution is governed by whether maximum accuracy is required at the upper, lower or centre part of the calibration range.

For solutions that do not obey the Beer - Lambert law, the concentration range used to determine the slope is now governed by the change in slope in addition to the accuracy of the absorbancy measurement.

At absorbancy differences less than 0·20, the accuracy of the determination decreases rapidly. A concentration difference of 4 g of uranium per litre was therefore used to determine the slope, and the system of plotting averages was applied to determine the slope at any specific uranium concentration.

When the absorbancy of the test solution is less than that of the reference solution, measurements are made by balancing the instrument at zero setting against the test solution, and calibration graphs constructed in this manner show zero absorbancy at the concentration of the reference solution. Milner and Phennah[10] have shown in detail that positive and negative absorbancies must be taken into account when corrections are applied and also when calculating the concentration of the test solution. Seven solutions were prepared to cover the range of 28 to 52 g of uranium per litre. A slit width was chosen (0·8 mm) such that, when the 52 g of uranium per litre solution was used to balance the instrument at zero,

Table III

Calibration data

Beckman spectrophotometer; 1-cm cells; slit width 0·8 mm; 430 mμ; 4 *M* sulphuric acid; temperature 23° C; b_1—reference cell = 1 cm

Uranium concentration, g per litre	$C_2 - C_1$ g per litre	R_1	R_2	R_3	R_4	$R_1 - R_3$	$R_2 - R_4$	$\frac{R_1 + R_3}{b_1 + b_2}$	$\frac{R_2 + R_4}{b_1 + b_2}$	$(A_2 - A_1)$ for single reference solution	Factor $\frac{C_2 - C_1}{A_2 - A_1}$	$(A_2 - A_1)$ for subsidiary reference solution	Factor $\frac{C_2 - C_1}{A_2 - A_1}$	Absorbancy referred to water	Calculated $R_1 - R_3$	Calculated $R_2 - R_4$
28	− 12	0·551	0	0·563	0	− 0·012	—	0·5561	—	− 0·5561	21·58	—	—	1·320	− 0·0130	—
30	—	—	—	—	—	—	—	—	—	—	—	− 0·1877	21·31	—	—	—
32	− 8	0·363	0	0·375	0	− 0·012	—	0·3684	—	− 0·3684	21·72	—	—	1·508	− 0·0136	—
34	—	—	—	—	—	—	—	—	—	—	—	− 0·1867	21·42	—	—	—
36	− 4	0·175	0	0·189	0	− 0·014	—	0·1817	—	− 0·1817	22·01	—	—	1·694	− 0·0142	—
38	—	—	—	—	—	—	—	—	—	—	—	− 0·1817	22·01	—	—	—
40	—	0	+ 0·0075	0	− 0·0075	—	+ 0·015	—	0	0	—	—	—	1·876	—	+ 0·0148
42	—	—	—	—	—	—	—	—	—	—	—	+ 0·1757	22·77	—	—	—
44	+ 4	0	0·183	0	0·169	—	+ 0·014	—	0·1757	+ 0·1757	22·77	—	—	2·052	—	+ 0·0154
46	—	—	—	—	—	—	—	—	—	—	—	+ 0·1657	24·14	—	—	—
48	+ 8	0	0·350	0	0·334	—	+ 0·016	—	0·3414	+ 0·3414	23·43	—	—	2·216	—	+ 0·0159
50	—	—	—	—	—	—	—	—	—	—	—	+ 0·1508	26·52	—	—	—
52	+ 12	0	0·501	0	0·485	—	+ 0·016	—	0·4922	+ 0·4922	24·38	—	—	2·368	—	+ 0·0164

Table III A

Calculation of cell errors

Uranium concentration, g per litre	Absorbancy (referred to water)	R_1	R_2	R_3	R_4	$R_2 - R_4$	$b_2 - b_1$	E_1	Mean $b_2 - b_1$	Mean E_1
28	1·320	0	+ 0·0055	0	− 0·0055	+ 0·011	+ 0·0036	0·0011	—	—
40	1·876	0	+ 0·0075	0	− 0·0075	+ 0·015	—	0·0013	+ 0·0033	+ 0·0012
52	2·368	0	+ 0·0090	0	− 0·0090	+ 0·018	+ 0·0030	0·0012	—	—

a movement of the absorbancy dial of 0·001 resulted in a meter deflection of 1 division and, further, that the meter sensitivity control was capable of re-balancing the instrument when the 40 g of uranium per litre solution was used to re-balance the instrument. The absorbancy differences between the 40 g of uranium per litre solution and the other solutions were then found by means of the solution reversal system and the readings obtained are given in Table III. The absorbancy differences are shown plotted against the uranium concentration in Fig. 6, curve A. The reciprocal of the slope (calibration factor) was calculated for all differences with respect to the 40 g of uranium per litre solution. These values are shown plotted against the uranium concentration in Fig. 6, curve B. Any absorbancy difference can therefore be directly converted to uranium concentration from curve A or, preferably, the calibration factor can be obtained from curve B and the concentration difference calculated, addition or subtraction of this value from 40 giving the uranium concentration in the test solution in g per litre. For the very small differences obtained for the samples given in Table IV the factor at 40 g of uranium per litre is not critical and was approximated to 24 ($\pm$ 0·0005 A = $\pm$ 0·03 per cent. of uranium).

For sample solutions with uranium concentrations differing greatly from 40 g per litre, improved accuracy is attained by preparing a further reference solution with a uranium content almost identical with that of the sample solution. The absorbancy of the sample solution is then measured against this new reference solution. With this system the calibration factor is obtained by reference to curve C of Fig. 6, which is prepared from a consideration of the absorbancy differences of consecutive solutions used in the calibration experiments (see Table III). This curve is constructed by determining the calibration factor for each concentration range and plotting this value against the corresponding mean concentration value for the range.

Calculating the concentration of the sample solution—

Single reference solution—The absorbancy of the sample solution is obtained with respect to the 40 g of uranium per litre reference solution; the corresponding factor is then derived from the graph (Fig. 6, curve B) and the uranium concentration of the test solution is calculated by means of the following expression—

$$\text{Uranium concentration, g per litre} = 40{\cdot}0 \pm F_1 [A_2 - A_1],$$

where F_1 is the corresponding factor on curve B to $\pm [A_2 - A_1]$.

Subsidiary reference solution—The absorbancy of the test solution is obtained with respect to the subsidiary reference solution, the corresponding factor derived from the graph (Fig. 6, curve C) and the uranium concentration of the test solution is calculated by means of the following expression—

$$\text{Uranium concentration, g per litre} = C_1 \pm F_2 [A_2 - A_1],$$

where F_2 is the corresponding factor on curve C at $\frac{A_2 - A_1}{2}$ (reference C_1 = zero absorbancy).

Results

The results for the analysis of samples of U_3O_8 prepared from different sources and of a specimen of uranium metal are given in Table IV. They are based on the U_3O_8 prepared from AnalaR uranyl acetate as the reference standard taken as 100 per cent. stoicheiometric U_3O_8 and can be considered relative to each other. This reference source was chosen because of the availability of large amounts of material from which large volumes of the stock reference solutions could be prepared. The importance of employing as the reference a material with an accurately known content of the constituent being determined has been stressed by Neal.[11] All the samples reported in Table IV were, therefore, analysed spectrographically for 37 elements. The high purity U_3O_8 showed a positive value for total metallic impurities of less than 0·005 per cent. and none of the other samples gave a total impurity content greater than 0·02 per cent.

From an examination of the relative values for uranium reported in Table IV, it is seen that a maximum difference of 0·06 per cent. of uranium resulted and it is unlikely that this could be due to some impurity undetected in the spectrographic analysis. In addition, the result for the sample of uranium metal proved to be slightly greater than any value obtained for the oxide samples and these values suggested that the conversion to U_3O_8 by ignition at

a temperature of 850° C is not quite stoicheiometric. This view is supported by the results of recent work carried out by Brouns and Mills,[9] which became available after the completion of our study. These workers have investigated in some detail the conversion of the higher oxide of uranium, UO_3, to the stoicheiometric form of U_3O_8, and have shown that, depending on the source of the uranium oxide, only 99·94 to 99·98 per cent. of the theoretical yield is obtained by igniting at a temperature of 850° C, irrespective of the time of ignition. This corresponds closely to the oxide values derived from Table IV, in which the metal sample is considered as 100·00 per cent. uranium and leads to the recommendation that all oxides should be ignited to constant weight at 1000° C. It is claimed by Brouns and Mills that at this temperature a recovery of 100·00 per cent. (± 0·01 per cent.) is attained irrespective of the source of the oxide by igniting for 1 hour and that no further change in weight occurs up to a temperature of 1200° C.

The sample of U_3O_8 designated as prepared from uranyl acetate in Table IV was the same as that used to prepare the stock primary reference solution and was included with the other samples as a "control" sample. The values for the different series in the table were obtained on different days over a period of 1 week and the value obtained for the "control" shows that any instability in the stock primary reference solution was less than the detectability of the method. Satisfactory control of the acidity and other factors had, therefore, been attained during sample analysis.

Table IV

Results obtained on samples of U_3O_8 and a sample of uranium metal

Beckman spectrophotometer; 1-cm cells; slit width 0·8 mm; 430 mμ; 4 *M* sulphuric acid; temperature 3° C

Source of uranium	Series 1	Series 2	Series 3	Mean percentage of uranium	Standard deviation from mean percentage of uranium	Coefficient of variation, %
Primary reference solution (U_3O_8 prepared from uranyl acetate)	100·00	100·00	100·00	100·00	nil	nil
U_3O_8 (high purity)	99·97	100·06	100·00	100·01	±0·046	±0·046
U_3O_8 (acetate)	100·03	100·00	99·97	100·00	±0·030	±0·030
U_3O_8 (Specpure)	100·00	100·00	99·97	99·99	±0·017	±0·017
U_3O_8 (UO_3)	100·00	99·97	99·94	99·97	±0·030	±0·030
U (metal as cast)	100·00	100·06	100·03	100·03	±0·030	±0·030

Over-all coefficient of variation = ±0·031 per cent.

Conclusions

The accuracy attained by the described procedure compares favourably with that attainable by volumetric or gravimetric methods. Moreover, this procedure is more specific. The need to use accurately calibrated volumetric equipment and to place solutions in thermostatically controlled baths is troublesome when only occasional samples are required to be analysed. The differential spectrophotometric technique is best suited to laboratories having to determine the uranium content of uranium-base materials continuously, since the time taken in setting up apparatus and calibrating equipment is then time spent advantageously. In addition, the technique is rapid in operation and is therefore convenient for control analysis.

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The Determination of Magnesium Oxide in Magnesium

BY H. J. ALLSOPP

Magnesium metal is removed from any oxide present by sublimation in vacuum and the residue is dissolved in dilute hydrochloric acid. Any iron or aluminium present is removed as the hydroxide, and the magnesium content of the residue is determined volumetrically by using disodium ethylenediaminetetra-acetate.

THE discovery of remarkable mechanical properties, especially at elevated temperatures, in extrusions made from fine flake powder of aluminium (S.A.P.) by Swiss workers[1] opened up a new field of powder metallurgy, which has now been extended to extrusions made from fine magnesium powder.[2] In these products no attempt is made to remove the natural oxide film present on the surface of each powder particle, and indeed, it is the included oxide films in the extruded material that by hindering grain growth, impart the high strength.

The oxide content is not a direct measure of the grain size (and therefore strength) of a powder extrusion, since a given oxide content may arise from a coarse powder heavily oxidised, or from a finer powder bearing the minimum film formed in air at room temperature.[3] Nevertheless, for development of this type of material, knowledge of the oxide content of any given grade of powder and extruded product is essential. It was desired, therefore, to devise a method for the determination of the oxide content of magnesium, and since chemical methods did not offer a likely field for investigation, the possibility of separating the metal from the oxide by vacuum distillation was explored.

EXPERIMENTAL

The first attempts at a separation of the magnesium oxide from magnesium were based on the well known method for the determination of oxide in aluminium, *i.e.*, dissolution in bromine - methanol. These failed owing to the solubility of magnesium oxide in the solvents used. Since no other likely chemical method could be envisaged, it became apparent that a gaseous separation would have to be employed.

A method has been described[4] in which the magnesium content of aluminium alloys is determined by vacuum distillation and another[5] wherein magnesium scrap is refined by vaporisation at extremely low pressure. It was thought likely that a separation could be effected along these lines.

The apparatus used, shown in Fig. 1, consisted of a platinum-wound electric furnace, which could be rolled back from a silica tube closed at one end and containing the samples to be processed. The open end of the silica tube was sealed with wax into a brass adaptor containing glass-wool as a filtering medium, the other end of the adaptor being sealed with wax into the low-pressure side of a conventional type of oil-diffusion pump. The high-pressure side of the diffusion pump was backed by a two-stage rotary oil pump. A Pirani gauge head was fitted between the diffusion and backing pumps and an ionisation gauge on the low-pressure side of the diffusion pump.

The samples under examination were placed in small mild-steel crucibles and covered with closely fitting lids carrying small holes as gas vents. As a precaution, the lids were held on with wire.

The first experiments consisted in placing a quantity of ignited magnesium oxide in a hole in a small block of pure magnesium and closing the hole with a small screw made from the same material. This was placed in the crucible, which was then put into the silica tube; the system was evacuated to approximately 10^{-4} mm of mercury and the temperature was raised to about 1000° C. On cooling, it was found that the residue of magnesium oxide had retained the shape of the hole in which it was placed in the sample and had not, as was originally expected, mixed with the molten magnesium metal. Hence this procedure did not simulate the conditions existing in a specimen of oxidised metal.

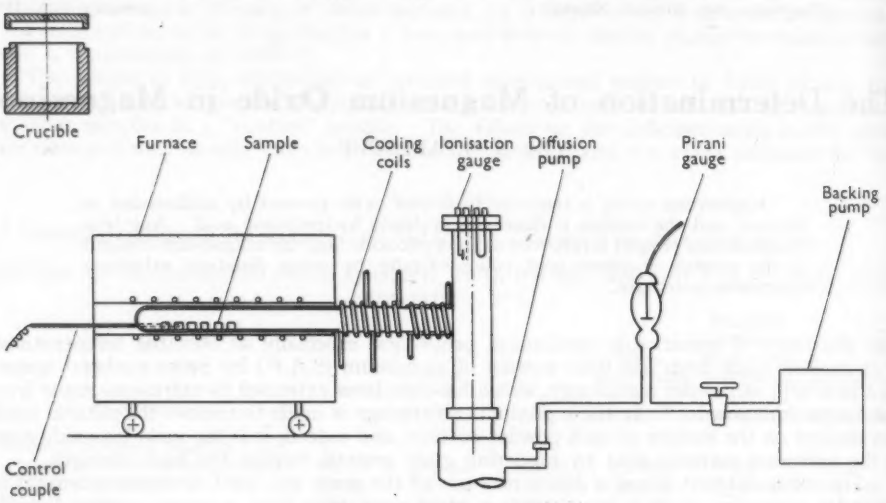

Fig. 1. Layout of apparatus

Three cold-compacts were then made from magnesium powder that had been well mixed, and these were distilled. The results are shown in Table I.

TABLE I

FIRST ATTEMPTS AT DETERMINATION OF OXIDE IN MAGNESIUM POWDER

Weight of compact, g	2·0133	2·0259	2·0047
Weight of residue, g	0·0310	0·0289	0·0290
Residue (magnesium oxide + impurities), %	1·54	1·44	1·45

On examination, the residue was found to be dark-grey in colour, probably owing to impurities in the original sample. Spectrographic examination of the ash showed the presence of traces of zinc, iron, silica, manganese, nickel and aluminium. It was therefore decided to determine the magnesium content of the residue chemically and thus to obtain a more accurate figure for the oxide content.

Eight cold-compacts were made from the same magnesium powder as before and vacuum distilled. The magnesium contents of the residues were determined by the well known phosphate method. Table II shows the results, which are in good agreement.

TABLE II

OXIDE DETERMINATION IN MAGNESIUM POWDER (PHOSPHATE FINISH)

Weight of compact, g	2·0100	2·0272	2·0350	2·0238	2·0226	2·0306	2·0308	1·9976
Weight of residue, g	0·0278	0·0284	0·0286	0·0296	0·0298	0·0297	0·0301	0·0300
Residue (magnesium oxide + impurities), %	1·39	1·40	1·41	1·46	1·47	1·46	1·48	1·50
Weight of magnesium pyrophosphate, g	0·0740	0·0735	0·0748	0·0751	0·0747	0·0750	0·0754	0·0754
Magnesium oxide, %	1·33	1·31	1·33	1·34	1·34	1·34	1·35	1·36

It was noticed that in all experiments, the residues from the vacuum distillations had retained the shape of the original compacts, thus showing that the magnesium metal had passed from the solid to the gaseous phase without liquifying, and therefore losses would not be caused by spattering on boiling.

In order to prove that the method was reliable it was necessary to make an addition of oxide and so establish the recovery. Zinc oxide was chosen, as it reacts readily with hot magnesium to give magnesium oxide and zinc, and it had already been proved that zinc distils over together with magnesium in vacuum.[4]

Table IIIA shows the magnesium oxide contents found on six cold-compacts of a new batch of magnesium powder that was used as a basis for the oxide addition. For these tests twelve 2-g portions of this material were weighed out and to six of these 0·1 g of zinc oxide was added, and to the remainder 0·2 g. The powders were mixed with a spatula, quantitatively compacted and vacuum distilled.

The magnesium in the residues was determined volumetrically by using disodium ethylenediaminetetra-acetate (EDTA), according to the method outlined by Banks.[6] The results obtained are given in Tables IIIB and IIIC and show good recovery.

Table IIIA

Magnesium powder used as basis for oxide addition

Weight of compacts, g	2·0032	2·0000	2·0055	2·0044	2·0089	2·0055
	Residues dissolved, diluted to 500 ml, and 100 ml taken					
Volume of EDTA solution (1 ml ≡ 0·0004 g of MgO), ml	15·1	15·1	14·9	14·9	15·0	15·1
Magnesium oxide, %	1·51	1·51	1·49	1·49	1·49	1·51

Table IIIB

Recovery of magnesium oxide after 0·1-g additions of zinc oxide

Residues dissolved, diluted to 500 ml, and 50 ml taken
1 ml of EDTA ≡ 0·0004 g of MgO

Weight of magnesium taken (1·5% of MgO), g	Weight of zinc oxide added, g	Total weight of sample, g	Weight of magnesium oxide from magnesium powder, g	Weight of magnesium oxide from zinc oxide, g	Magnesium oxide calculated on total sample, g	Magnesium oxide calculated on total sample, %	Volume of EDTA used, ml	Magnesium oxide recovered on total sample, %
2·0021	0·1004	2·1025	0·0300	0·0497	0·0797	3·79	20·1	3·83
2·0010	0·1027	2·1037	0·0300	0·0509	0·0809	3·85	20·2	3·84
2·0003	0·1000	2·1003	0·0300	0·0496	0·0796	3·79	19·9	3·79
1·9995	0·1060	2·1055	0·0300	0·0525	0·0825	3·92	20·7	3·93
2·0017	0·1004	2·1021	0·0300	0·0497	0·0797	3·79	19·8	3·77
2·0011	0·1100	2·1111	0·0300	0·0545	0·0845	4·00	21·1	4·00

Table IIIC

Recovery of magnesium oxide after 0·2-g additions of zinc oxide

Residues dissolved, diluted to 500 ml, and 50 ml taken
1 ml of EDTA ≡ 0·0004 g of MgO

Weight of magnesium taken (1·5% of MgO), g	Weight of zinc oxide added, g	Total weight of sample, g	Weight of magnesium oxide from magnesium powder, g	Weight of magnesium oxide from zinc oxide, g	Magnesium oxide calculated on total sample, g	Magnesium oxide calculated on total sample, %	Volume of EDTA used, ml	Magnesium oxide recovered on total sample, %
2·0027	0·2038	2·2065	0·0300	0·1009	0·1309	5·93	32·7	5·93
2·0076	0·2006	2·2082	0·0301	0·0994	0·1295	5·87	32·2	5·84
2·0019	0·2023	2·2042	0·0300	0·1002	0·1302	5·91	32·7	5·94
2·0035	0·2065	2·2100	0·0300	0·1023	0·1323	5·98	33·2	6·01
2·0070	0·2013	2·2083	0·0301	0·0997	0·1298	5·88	32·5	5·89
2·0047	0·2012	2·2059	0·0301	0·0997	0·1298	5·88	32·65	5·92

The nitrogen content of residues from six distillations was determined and figures showing between 0·06 and 0·08 per cent. of nitrogen were obtained. This indicated that less than 0·2 per

cent. of the magnesium in the residue was present as nitride and, since its effect on the final result was negligible, its presence was ignored.

TABLE IV

TYPICAL RESULTS OBTAINED BY RECOMMENDED METHOD

Sample No. 1—						
Weight of sample, g	3·8268	3·8441	3·8394	3·8484	3·8408	3·8280
	Residues dissolved, diluted to 500 ml, and 50 ml taken					
Volume of EDTA solution (1 ml ≡ 0·000399 g of MgO), ml	10·95	11·45	11·35	11·50	11·55	11·55
Magnesium oxide, %	1·14	1·18	1·18	1·19	1·20	1·20
Sample No. 2—						
Weight of sample, g	2·3817	2·3947	2·3771	2·3443	2·3688	2·3730
	Residues dissolved, diluted to 500 ml, and 100 ml taken					
Volume of EDTA solution (1 ml ≡ 0·000399 g of MgO), ml	26·95	27·45	27·20	26·85	27·10	27·65
Magnesium oxide, %	2·25	2·28	2·28	2·28	2·28	2·32
Sample 240 *Fines*—						
Weight of sample, g	2·4553				2·4779	
	Residues dissolved, diluted to 500 ml, and 100 ml taken					
Volume of EDTA solution (1 ml ≡ 0·000399 g of MgO), ml	23·8				24·2	
Magnesium oxide, %	1·93				1·95	
Sample S—						
Weight of sample, g	1·4838				1·4281	
	Residues dissolved, diluted to 250 ml, and 100 ml taken					
Volume of EDTA solution (1 ml ≡ 0·000399 g of MgO), ml	20·8				20·5	
Magnesium oxide, %	1·40				1·43	
*Sample U*1—						
Weight of sample, g	1·5650				1·4995	
	Residues dissolved, diluted to 500 ml, and 100 ml taken					
Volume of EDTA solution (1 ml ≡ 0·000399 g of MgO), ml	18·5				17·8	
Magnesium oxide, %	2·36				2·37	

METHOD

REAGENTS—

Disodium ethylenediaminetetra-acetate (*EDTA*), *approximately* 0·02 N—The solution should be standardised against ignited magnesium oxide before use.

*Solochrome black W.D.F.A. indicator**—Make a 0·5 per cent. w/v solution in a mixture of equal parts by volume of the purest available triethanolamine and *iso*propanol.

Ammonium chloride, 25 per cent. w/v solution.

PROCEDURE—

Place a suitable weight of sample previously cleaned of surface oxide into a mild-steel crucible and keep the perforated lid firmly in position by means of wire. Insert into the silica tube and connect to the diffusion pump. Evacuate the system to about 10^{-4} mm of mercury. Slowly raise the temperature to 900° to 1000° C over a period of 2 hours. Roll the furnace away from the silica tube and allow the tube to cool to room temperature.

Remove the crucible from the apparatus and transfer the residue to a beaker.

Add 10 ml of water, 10 ml of concentrated hydrochloric acid and 10 drops of concentrated nitric acid, and evaporate until almost dry. Dilute with about 10 ml of water, add 20 ml of 25 per cent. ammonium chloride solution, a portion of litmus paper and render just ammoniacal. Add two drops of ammonia solution, sp.gr. 0·880, in excess. Bring just to the boil.

Filter on a No. 41 Whatman filter-paper and wash the precipitated hydroxides of iron and aluminium with water. Redissolve the precipitate into the original beaker, using 10 ml

* Solochrome black W.D.F.A., manufactured by Imperial Chemical Industries Limited, is the equivalent of Eriochrome black T.

of 20 per cent. hydrochloric acid. Re-precipitate the hydroxides, using another 20 ml of 25 per cent. ammonium chloride solution. Bring just to the boil.

Filter and wash as before and combine the filtrates in a 500-ml calibrated flask. Make up to the mark and mix well.

Transfer a suitable aliquot to a conical flask, dilute to 100 ml with distilled water (if necessary) and add ammonia solution, sp.gr. 0·880, in accordance with the following table—

Volume of aliquot, ml..	20	50	100
Volume of ammonia solution, sp.gr. 0·880, ml ..	4	6	10

Add 10 drops of indicator and titrate with EDTA solution to a blue end-point without any tinge of red.

Sometimes it may be necessary to dilute the bulk solution to 250 ml or less, in which event the volumes of the additions of 25 per cent. ammonium chloride solution should be adjusted accordingly.

Results

Some typical values for various samples are given in Table IV.

Conclusions

The magnesium oxide content of magnesium metal can be obtained satisfactorily by sublimation of the metal in vacuum followed by the determination of the magnesium in a solution of the residue by titration with disodium ethylenediaminetetra-acetate.

The time taken for a single determination is in the region of 5 hours.

I thank Mr. T. R. F. W. Fennell, who determined the nitrogen content of the residues.
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Determination of Mercury in Fungicidal Preparations Containing Organo-mercury Compounds

Part I. The Determination of Organo-mercury Compounds by Direct Titration Procedures

By K. F. SPOREK

Several analytical procedures for the determination of mercury in the form of organo-mercury compounds are described. Details are given of methods suitable for the direct titration of organo-mercury compounds with the organic radical attached to the mercury atom.

The expanding use of mercury compounds, especially those with organically combined mercury, for fungicidal purposes has led to a large and constantly growing number of preparations being sold on the market. These materials are used by farmers and nurserymen for the treatment of seeds, bulbs, tubers, plants, fruit trees and so on.

The range of mercury compounds used as fungicides is wide and comprises inorganic and organic mercury derivatives. The formulations containing mercury are also numerous and vary in the character of the mercury compound, its content, the presence or absence of other active agents in the form of insecticides (hexachloro*cyclo*hexane, dieldrin, lead arsenate) and the type of the inert diluent (filler), which may be soluble or insoluble in water. When account is taken of the large number of possible diluents that may be employed and the number of mercury compounds with or without other active or inactive agents, it is easy to imagine the complexity of the final products.

The routine analysis of the mercury fungicides normally is limited to the determination of total mercury and only rarely is the determination of other constituents attempted. The determination of total mercury is, however, on its own a difficult problem owing to the following facts—

(1) Mercury compounds are volatile, and this calls for special precautions when treatment at temperatures above room temperature is applied. Ethylmercuric chloride, for example, even at room temperature is sufficiently volatile to make itself detectable by the smell and taste of its vapour.

(2) The difficulty in transforming organo-mercury compounds into inorganic mercury salts, which normally calls for rather drastic treatment, such as boiling with sulphuric - nitric acid mixture or sulphuric acid and potassium permanganate. In the course of such treatment other constituents of the material are also attacked.

(3) The fact that the best quantitative determination of mercury, which is by way of the thiocyanate titration in acid solution, with ferric alum as indicator, is seriously affected by even small amounts of halide ions. The titration itself is very accurate and simple, but as it must be conducted in complete absence of chloride ion this makes it less attractive.

(4) The mercury content of fungicidal preparations is low (1 to 2 per cent.) and so the size of a sample required for a determination is necessarily large (10 to 20 g). The fillers in such a large quantity of the sample usually contain enough chloride to make a titration with thiocyanate impossible.

As the mercury content of fungicides based on this element is important, not only in view of the high price of the active agent used but also because of dangers of damage to the treated seeds, trees and so on with an overdose of mercury compounds, it is of paramount importance to know the concentration of mercury in these preparations.

In the search for a method that would give accurate results, be applicable to as many different products as possible, be reasonably easy to carry out and not involve use of expensive and specialised reagents, several existing procedures for the determination of mercury were tried. The details of the tested methods as well as their usefulness when such was found are described in the following section.

Experimental

Determination of organo-mercury ion by direct titration with standard thiocyanate—

The titration of mercuric salts, usually in the form of nitrate or sulphate, with ammonium thiocyanate solutions, with ferric alum as indicator, is the most widely used procedure for the determination of this element. When organo-mercury compounds are to be tested they are usually transformed into the inorganic mercury salts by oxidation of the organic radical and then, provided chloride ion is absent, they are titrated with the thiocyanate reagent.

In this work an attempt was made to titrate certain organo-mercury compounds with thiocyanate without destroying the organic radical. Aqueous solutions of the compounds shown in Table I were titrated in the presence of nitric acid with 0·1 *N* ammonium thiocyanate solution, ferric alum being used as indicator.

Table I

Direct titration of organo-mercury compounds with standard 0·1 *N* ammonium thiocyanate

Compound	Solubility in 0·1 *N* nitric acid solution*	Remarks on titration
Ethylmercuric acetate	Soluble	Sharp end-point; precipitate
Methoxyethylmercuric acetate ..	Very soluble	Poor end-point; no precipitation
Phenylmercuric acetate	Soluble	Sharp end-point; precipitate formed, soluble in acetone
Methoxyethylmercuric borate ..	Very soluble	Poor end-point; no precipitation
Phenylmercuric borate	Sparingly soluble	Sharp end-point; precipitate formed, soluble in acetone
Phenylmercuric nitrate	Sparingly soluble	Sharp end-point; precipitate formed, soluble in acetone
Bis(ethoxyethylmercuric) hydrogen phosphate	Sparingly soluble	Sharp end-point; no precipitation
Ethylmercuric phosphate	Soluble	Sharp end-point; precipitate formed, soluble in acetone
Hydroxyethylmercuric silicate ..	Sparingly soluble	Sharp end-point; no precipitation
Methoxyethylmercuric silicate ..	Soluble	Sharp end-point; no precipitation
Methoxyethylmercuric tannate ..	Soluble	Reacts with ferric alum
Sodium ethylmercurithiosalicylate ..	Soluble	Sharp end-point, no precipitation
Phenylmercuric urea	Sparingly soluble	Sharp end-point; precipitate formed, insoluble in acetone

* "Soluble" indicates 1 per cent. or greater solubility.

The results in Table I show that many organo-mercury compounds can be directly titrated with a standard thiocyanate solution. The end-point was usually of the same quality as that for inorganic mercury compounds. Some organo-mercury compounds formed sparingly soluble thiocyanates, which were precipitated during the titration. This precipitation did not interfere with the end-point or affect the result, and all the precipitates were easily soluble on addition of acetone to the titrated solution. The factor for the thiocyanate solution was twice the value applicable when the reagent was used for the titration of inorganic mercury salts (1 ml of 0·1 *N* solution ≡ 0·02006 g of mercury for organo-mercury compounds).

Determination of organo-mercury compounds by non-aqueous titration—

The procedure tested had originally been used for the determination of ethylenic compounds,[1] which were treated with mercuric acetate in the presence of methanol. The reaction product for ethylene was methoxyethylmercuric acetate. The titration, which determines only the ionised mercury, gave a measure of the amount of mercuric acetate left in the solution plus the valency occupied by the acetate radical in methoxyethylmercuric acetate. The acetate radical was titrated with standard hydrochloric acid in a solvent mixture containing chloroform and propylene glycol. The end-point of this titration was extremely sharp and for this reason the procedure was tested for its suitability for the determination of certain organo-mercury compounds.

The details of the procedure were as follows—About 0·5 g of the compound was dissolved in 25 ml of a chloroform - propylene glycol mixture (1 + 1) contained in a 100-ml conical flask, 5 drops of 0·2 per cent. thymol blue indicator in ethanol were added, and the solution

was titrated with a 0·1 *N* hydrochloric acid solution (made by dissolving 9 ml of concentrated acid in 1 litre of the solvent mixture). The end-point was indicated by a sharp change of colour from yellow to pink (1 ml of 0·1 *N* hydrochloric acid ≡ 0·02006 g of mercury in organo-mercury compounds).

Table II shows the compounds tested and the results obtained. From this table it is seen that many organo-mercury compounds are titratable under the conditions described. The end-points were usually sharp to 0·01 to 0·02 ml of the reagent in about 50 ml of the

TABLE II

DETERMINATION OF ORGANO-MERCURY COMPOUNDS BY DIRECT TITRATION IN NON-AQUEOUS MEDIUM

Compound	Solubility in the solvent mixture*	Remarks on titration
Ethylmercuric acetate	Very soluble	Good end-point
Methoxyethylmercuric acetate	Very soluble	Good end-point
Phenylmercuric acetate, pure	Very soluble	Good end-point
Phenylmercuric acetate, technical	Very soluble	Good end-point
Mercuric acetate	Sparingly soluble	Good end-point
Methoxyethylmercuric borate	Very soluble	Good end-point
Phenylmercuric borate	Very soluble	Poor end-point, but possible to titrate
Methoxyethylmercuric chloride	Soluble	Not titratable
Phenylmercuric nitrate	Insoluble	Not titratable
Bis(ethoxyethylmercuric) hydrogen phosphate	Insoluble	Good end-point when titrated in hot solution
Ethylmercuric phosphate	Insoluble	Not titratable
Ethoxyethylmercuric silicate	Insoluble	Good end-point when titrated in hot solution
Hydroxyethylmercuric silicate	Insoluble	Good end-point when titrated in hot solution
Methoxyethylmercuric silicate	Insoluble	Good end-point when titrated in hot solution
Methoxyethylmercuric tannate	Insoluble	Good end-point when titrated in hot solution
Sodium ethylmercurithiosalicylate	Very soluble	Good end-point
Phenylmercuric urea	Insoluble	Good end-point when titrated in hot solution

* "Soluble" indicates 1 per cent. or greater solubility.

TABLE III

DETERMINATION OF ORGANO-MERCURY COMPOUNDS BY DIRECT TITRATION WITH STANDARD ACID

Compound	Solubility in the reaction mixture*	Remarks on titration
Ethylmercuric acetate	Soluble	Good end-point; precipitate with KI
Methoxyethylmercuric acetate	Very soluble	Good end-point
Phenylmercuric acetate	Soluble	Good end-point; precipitate with KI
Mercuric acetate	Soluble	Good end-point; precipitate with KI
Methoxyethylmercuric borate	Very soluble	Good end-point
Phenylmercuric borate	Soluble	Good end-point; precipitate with KI
Ethylmercuric chloride	Soluble	Poor end-point; precipitate with KI
Ethoxyethylmercuric chloride	Soluble	Good end-point
Hydroxyethylmercuric chloride	Soluble	Good end-point
Methoxyethylmercuric chloride	Soluble	Good end-point
Phenylmercuric chloride	Soluble	Good end-point; precipitate with KI
Phenylmercuric nitrate	Sparingly soluble	Good end-point; precipitate with KI
Bis(ethoxyethylmercuric) hydrogen phosphate	Insoluble	Not titratable
Ethylmercuric phosphate	Sparingly soluble	Good end-point; precipitate with KI
Ethoxyethylmercuric silicate	Insoluble	Not titratable
Hydroxyethylmercuric silicate	Insoluble	Not titratable
Methoxyethylmercuric silicate	Insoluble	Not titratable
Methoxyethylmercuric tannate	Insoluble	Not titratable
Sodium ethylmercurithiosalicylate	Soluble	Not titratable
Phenylmercuric urea	Sparingly soluble	Good end-point; precipitate with KI

* "Soluble" indicates 1 per cent. or greater solubility.

final solution. Sparingly soluble compounds were titrated at the boiling point of the solvent mixture and near the end-point the reagent was added dropwise.

DETERMINATION OF ORGANO-MERCURY COMPOUNDS BY TITRATION WITH STANDARD ACID—

A procedure described in the literature[2,3] and based on the fact that solutions of mercuric oxide yield hydroxyl ion when treated with potassium iodide was stated to be unaffected by the chloride ion. In this work the method was tested on organo-mercury compounds. About 0·5 g of the sample was dissolved in 50 ml of 50 per cent. aqueous acetone, 3 g of urea were added, followed by a few drops of phenolphthalein indicator and 0·1 *N* sodium hydroxide solution until the colour turned to faint pink. The mixture was then treated with 5 g of potassium iodide, and the liberated alkali was titrated with 0·1 *N* perchloric acid solution to the same faint pink colour of the indicator.

The compounds tested and the results obtained are shown in Table III, from which it is seen that organo-mercury chlorides are amongst the compounds titratable under the described conditions. This is the only procedure capable of determining this type of compound and is therefore especially useful for rapid determinations of phenylmercuric chloride, ethylmercuric chloride and ethoxyethylmercuric chloride, which are amongst the most commonly used organo-mercury fungicides.

CONCLUSIONS

The procedures described above involving direct titrations of organo-mercury compounds are especially useful in assessing the purity of raw materials and testing mixtures from which these compounds can easily be extracted with a suitable solvent.

The thiocyanate titration will account for almost all types of compounds except the chlorides, the non-aqueous titration for all organo-mercury salts of weak acids and direct titration with acid for almost all soluble compounds, including chlorides. The various combinations of the above procedures are capable therefore of dealing with any type of compound. Singly, however, none of them could be applied to the testing of the extremely wide range of organo-mercury fungicides and so their usefulness in this respect is limited.

I express my thanks to Plant Protection Ltd., for permission to publish this paper, to J. M. Winchester, Chief Chemist, for his helpful criticism and to Miss A. Butler for assistance in the experimental work.

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TECHNICAL DEPARTMENT
PLANT PROTECTION LIMITED
YALDING, KENT

March 7th, 1956

Determination of Mercury in Fungicidal Preparations Containing Organo-mercury Compounds

Part II. The Determination of Organo-mercury Compounds after Decomposition to Mercuric Sulphide

By K. F. SPOREK

A new technique based on the formation of water-soluble complexes of certain organo-mercury compounds with sodium sulphide is described. This technique was found suitable for the determination of mercury in complex commercial preparations containing various organo-mercury compounds, fillers, chlorinated organic insecticides, dyes, pigments and so on.

THE fact that the direct titration procedures described in Part I, although useful for some purposes, were of rather limited value for testing the wide range of organo-mercury fungicides, suggested that it would usually be necessary to separate the organo-mercury compound from the rest of the material (insoluble fillers, organo-chlorine insecticides, dyes, pigments, oils, etc.) and then carry out the determination on the extract.

Experiments were therefore carried out in order to find a suitable medium for this purpose.

EXPERIMENTAL

A number of solvents and solutions of compounds known to form complexes with mercury was tested in order to find one that would be suitable for extracting organo-mercury compounds from fungicidal preparations. After several attempts it was observed that concentrated sodium sulphide solutions had extraordinary solvent properties for organo-mercury compounds. Complete dissolution could usually be effected by shaking the compound with an excess of the aqueous sulphide. Water-soluble mercury compounds on treatment with the reagent would first produce an insoluble organo-mercury sulphide, which redissolved in an excess of the sodium sulphide solution.

The compounds tested and the relative ease in getting them into solution are shown in Table I. At this point it may be appropriate to note that organo-mercury compounds could be divided into the following four groups, according to their solubility—

(*i*) compounds soluble in organic solvents (phenylmercuric chloride),
(*ii*) compounds soluble in organic solvents and in water (phenylmercuric acetate),
(*iii*) compounds very soluble in water (methoxyethylmercuric acetate), and
(*iv*) compounds insoluble in organic solvents and in water (ethoxyethylmercuric silicate).

The fact that all these types of organo-mercury compounds were easily soluble in the sodium sulphide reagent made it possible to devise a general procedure for their quantitative extraction from fungicidal preparations. The sulphide reagent had no solvent action on the chlorinated organic insecticides, oils, pigments and dyes usually found in these materials. The extracts were therefore free from the substances that in further stages of this determination would yield large quantities of chloride ion. The extraction of organo-mercury compounds is expedited by heating the suspension with the sulphide reagent and so this procedure was finally adopted.

A further observation was the easy decomposition of organo-mercury compounds to inorganic mercury sulphide when the extracts were acidified with dilute sulphuric acid. This decomposition was also affected by the temperature of the solution and proceeded at different rates with different organo-mercury compounds.

Sulphide solutions of phenylmercuric compounds when acidified in the cold first produced a white precipitate (phenylmercuric sulphide), which on standing at room temperature slowly darkened and after a few hours was completely changed into the inorganic mercury sulphide. The decomposition proceeded rapidly when the same operation was performed in a hot solution. On boiling the solution for a few minutes all organo-mercury sulphides were quantitatively transformed into mercuric sulphide, which could be filtered off and

either weighed or after oxidation titrated with thiocyanate. These facts led to the development of a method for determining total mercury in a wide range of products containing organo-mercury compounds.

In the technique finally adopted a 15 per cent. aqueous sodium sulphide solution (nearly saturated solution) is used for extracting the organo-mercury compound from the tested

TABLE I

SOLUBILITIES OF ORGANO-MERCURY COMPOUNDS IN 15 PER CENT. SODIUM SULPHIDE (Na_2S) SOLUTIONS

Compound	Solubility in water	Solubility in excess of sodium sulphide	Behaviour on acidification with dilute sulphuric acid
Ethylmercuric acetate ..	Insoluble	Soluble	White precipitate rapidly changing to black (HgS) on heating
Methoxyethylmercuric acetate	Very soluble	Very soluble	White precipitate changing to black in cold
Phenylmercuric acetate ..	Sparingly soluble	Very soluble	White precipitate changing to black on boiling
Mercuric acetate	Soluble	Very soluble	Black precipitate
Methoxyethylmercuric borate	Very soluble	Very soluble	White precipitate changing slowly to black in cold
Phenylmercuric borate ..	Insoluble	Soluble	White precipitate changing to black on boiling
Ethylmercuric chloride ..	Insoluble	Soluble	White precipitate changing to black on boiling
Ethoxyethylmercuric chloride	Insoluble	Very soluble	Black precipitate
Hydroxyethylmercuric chloride	Soluble	Very soluble	Black precipitate
Methoxyethylmercuric chloride	Soluble	Very soluble	Black precipitate
Phenylmercuric chloride..	Insoluble	Soluble	White precipitate changing to black on boiling
Phenylmercuric nitrate ..	Insoluble	Soluble	White precipitate changing to black on boiling
Bis(ethoxyethylmercuric) hydrogen phosphate ..	Insoluble	Soluble	Black precipitate
Ethylmercury phosphate	Insoluble	Soluble	White precipitate changing to black on boiling
Ethoxyethylmercuric silicate	Insoluble	Soluble	Black precipitate
Hydroxyethylmercuric silicate	Insoluble	Sparingly soluble in cold	Black precipitate
Methoxyethylmercuric silicate	Insoluble	Soluble	Black precipitate
Methoxyethylmercuric tannate	Insoluble	Soluble	Black precipitate
Sodium ethylmercuric thiosalicylate	Sparingly soluble	Very soluble	White precipitate changing to black on boiling
Phenylmercuric urea ..	Insoluble	Soluble	White precipitate changing to black on boiling

* "Soluble" indicates 1 per cent. or greater solubility.

material. The mixture is filtered to remove the insoluble matter comprising mainly inert diluents and chlorinated organic insecticides, and the filtrate is heated and then acidified with sulphuric acid. The precipitated mercuric sulphide is filtered off and either weighed or titrated after oxidation with a mixture of sulphuric acid and potassium nitrate. The latter procedure is, however, preferable, owing to its higher specificity for mercury.

The advantages in going through the above set of operations are—

(1) The separation of organo-mercury compounds from inert diluents such as china clay, talc and chalk, and from insecticides such as hexachloro*cyclo*hexane and dieldrin, which are incorporated in some dual-purpose fungicide preparations. Many dyes, pigments and oils are also insoluble in the aqueous reagent. The

extract therefore contains the mercury compounds in a comparatively pure state. When the diluents are soluble in water, *e.g.*, sodium carbonate or sodium bicarbonate, the sulphide reagent ensures complete dissolution of the organo-mercury compound (most organo-mercury compounds are sparingly soluble in water) so that subsequent precipitation of the sulphide is carried out from homogeneous solution.

(2) On acidification of the solution containing the organo-mercury compound only mercuric sulphide is precipitated; it is easy to filter off and wash free from any traces of organo-chlorine compounds and chlorides and so after oxidation it gives a solution suitable for the thiocyanate titration. This makes the procedure specific for mercury.

(3) The procedure makes it possible to determine organo-mercury compounds in solutions containing as little as 0·01 per cent. of mercury, owing to the very high insolubility and non-volatility of mercuric sulphide.

Two procedures were finally devised, one suitable for materials containing organo-mercury compounds in water-soluble diluents, the other for materials made with water-insoluble diluents and containing organic insecticides, dyes, pigments, oils, etc. Fungicides containing the organo-mercury compound in an insoluble diluent and no other constituent could also be tested by the first procedure, which is simpler as it does not involve filtration of the sulphide solution. In adopting this step it is assumed, however, that the insoluble filler is washed free from any chloride-containing impurities during the filtration and washing of mercuric sulphide and will produce no chloride ion on subsequent treatment with the nitration mixture.

Method

Reagents—

All reagents should be of recognised analytical grade.

Sodium sulphide solution—Dissolve 50 g of sodium sulphide crystals, $Na_2S.9H_2O$, in 50 ml of water. If necessary, heat slightly to facilitate solution.

Sulphuric acid, diluted—Dilute 500 ml of concentrated sulphuric acid to 1 litre with water.

Sulphuric acid, concentrated.

Potassium nitrate, crystalline.

Potassium permanganate—A saturated aqueous solution.

Ferrous ammonium sulphate—A 5 per cent. aqueous solution.

Ferric alum indicator solution—Dissolve 140 g of ferric ammonium sulphate in 1 litre of water, and add sufficient nitric acid, sp.gr. 1·42, to change the colour of the solution from reddish-brown to yellow. Use 1 ml of this solution for every 50 ml of liquid.

Ammonium thiocyanate, 0·1 N—Weigh 8 g of ammonium thiocyanate for each litre of solution required, dissolve the salt in water, transfer the solution to a calibrated flask, dilute it to the mark, and shake it well. Standardise the solution against standard silver nitrate, using ferric alum as indicator.

Procedure 1—

Weigh into a 500-ml flask with a B34 ground-glass socket sufficient of the sample to provide 0·2 to 0·3 g of mercury and add 150 ml of water and 20 ml of sodium sulphide reagent. Attach an 18-inch long air-condenser with a B34 ground-glass cone, mix the contents of the flask well and heat the solution to boiling. Add slowly to the hot solution enough diluted sulphuric acid to produce a permanent precipitate, and then another 30 ml in excess and boil for 10 minutes. Filter the mixture with suction through a small glass crucible having a sintered-glass disc of porosity No. 3 and containing a thin pad of asbestos. Wash the flask and the precipitate with a small quantity of distilled water.

Place the crucible inside the flask, attach the air-condenser, add 10 g of potassium nitrate and 25 ml of concentrated sulphuric acid, mix well and boil until the evolution of brown fumes ceases. Cool the flask to room temperature, carefully dilute with water to about 150 ml and boil again to remove nitrous acid. Cool to room temperature, add an excess of saturated potassium permanganate solution and set aside for 5 minutes. Remove the excess with a few drops of ferrous ammonium sulphate solution, treat with 5 ml of ferric alum indicator and titrate with 0·1 *N* ammonium thiocyanate solution.

1 ml of 0·1 *N* ammonium thiocyanate ≡ 0·01003 g of mercury.

PROCEDURE 2—

Weigh into a 500-ml conical flask with a B34 ground-glass socket sufficient of the sample to provide 0·2 to 0·3 g of mercury, add 50 ml of sodium sulphide solution, mix the contents of the flask by swirling and then attach the 18-inch long air-condenser with a B34 ground-glass cone. Heat the flask to boiling while swirling constantly. Keep boiling for a few minutes and then dilute the mixture by pouring 150 ml of cold water through the condenser. Filter the mixture using suction through a sintered-glass filter funnel of porosity No. 2 or 3

TABLE II

DETERMINATION OF MERCURY IN ORGANO-MERCURY FUNGICIDES BY THE SULPHIDE METHOD

Sample	Mercury found, %	Remarks
By procedure 1—		
Phenylmercuric acetate - ethoxyethylmercuric silicate mixture (30 per cent. of mercury) on insoluble filler ..	29·1 29·0	29·2 by direct nitration and thiocyanate titration
Methoxyethylmercuric acetate, dilute aqueous solutions	0·0132 0·0245 0·0648 0·118 0·130 0·240 0·261 0·471 1·299 2·360	0·0130 theoretical 0·0237 by dilution 0·0650 0·118 0·130 0·237 0·260 0·473 1·300 2·370
Mercuric chloride, AnalaR, dried	100·1 99·9	
By procedure 2—		
Phenylmercuric acetate - ethoxyethylmercuric silicate mixture (3 per cent. of mercury) with γ-hexachloro-*cyclo*hexane (40 per cent.) on insoluble filler with oil, dye and pigments	3·45 3·42 3·43 3·45 3·50 3·45 3·46	3·48 theoretical
Phenylmercuric acetate - ethoxyethylmercuric silicate mixture (1·5 per cent. of mercury) on insoluble filler with oil and pigment	1·52	
Phenylmercuric acetate - ethylmercuric chloride mixture (2 per cent. of mercury) with γ-hexachloro*cyclo*hexane (40 per cent.) on insoluble filler with oil and pigment	2·23	2·24 theoretical
Phenylmercuric acetate - ethylmercuric chloride mixture (2 per cent. of mercury) with γ-hexachloro*cyclo*hexane (40 per cent.) on insoluble filler with oil, pigment and dye	1·94 1·95	
Phenylmercuric urea (1 per cent. of mercury) with γ-hexachloro*cyclo*hexane (40 per cent.) on insoluble filler with pigment and oil	1·04 0·95	
Phenylmercuric urea (4 per cent. of mercury) on insoluble filler, with oil and pigment	4·11 3·94 4·04 4·06	
Phenylmercuric chloride with dieldrin (40 per cent.) on insoluble filler	1·61 1·59 1·56 1·59 1·57 1·58	1·60 theoretical

provided with a thin asbestos pad and wash the residue with 50 ml of water. Transfer the filtrate quantitatively into a clean 500-ml Quickfit flask, B34 neck, attach an air-condenser, heat to boiling and add slowly enough diluted sulphuric acid to form a permanent precipitate,

then add another 30 ml in excess and boil for 10 minutes. Continue in exactly the same way as described for procedure 1.

Results

Many organo-mercury fungicides were tested by the above procedures and the results are shown in Table II. The range of products tested covers almost all types of this material made at the present time. The results are satisfactory, comparing well with the theoretical values and values obtained by other methods when available.

Discussion of results

The time required for a determination was about 1 hour for procedure 1 and 1½ to 2 hours for procedure 2. It was possible, however, to run two and even three experiments in parallel, with big savings in time per determination.

The acidification of the sodium sulphide extract and the nitration of the mercuric sulphide could be carried out in open vessels without loss of mercury by volatilisation. However, to avoid losses by spray during the evolution of hydrogen sulphide and heating the nitration mixture, the flasks were provided with air-condensers.

In the procedures described above the decomposition of the organo-mercury sulphides into mercuric sulphide was effected by treatment with dilute acid under comparatively mild conditions, which did not affect the organic radical apart from detaching it from the mercury atom. The same effect was achieved, however, by treatment with, for example, hydrogen peroxide, but in this case no hydrogen sulphide was produced. In this way benzene was easily detected by its smell when any of the phenylmercuric compounds was tested and organo-mercury compounds with aliphatic radicals produced mercaptans. Different organo-mercury compounds could therefore be identified and also easily distinguished from inorganic mercury.

Work on the above problem is still in progress and it is hoped to give a fuller account of it at some later date.

I express my thanks to Plant Protection Ltd., for permission to publish this paper, to J. M. Winchester, Chief Chemist, for his helpful criticism and to Miss A. Butler for assistance in the experimental work.

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The Determination of Non-fat Dry Milk Solids in Bread by Paper Chromatography

By H. ZENTNER

A method has been devised for the determination of non-fat dry milk solids in bread. The lactose, which usually comprises 50 per cent. of the non-fat dry milk solids, is separated from residual maltose by descending paper chromatography with an *n*-butanol - pyridine - water system containing silver nitrate. The optical densities of the silver spots are then determined in a densitometer. The concentration of lactose in the bread sample is read from a calibration graph prepared by running lactose solutions of the appropriate range of concentration on the same sheet of Whatman No. 1 filter-paper. Good results were obtained with laboratory baked loaves containing non-fat dry milk solids. The method eliminates the blank determination, which has proved a source of error in fermentation methods.

THE determination of non-fat dry milk solids in bread is generally based upon the principle of selective fermentation of the sugars present in the bread extract with bakers' yeast, which does not ferment lactose. The non-fat milk solids content is usually calculated as twice the lactose content.

In these methods it is necessary to determine the reducing power of a number of substances, other than lactose, that are not fermented by the yeast, and to deduct this blank from the total reducing power of the fermented bread extract. The blank is obtained by extracting a sample of bread containing no milk and treating it in the same way as the sample under investigation.[1]

There is no doubt that the reducing non-fermentable substances of the blank consist of dextrins and reducing polysaccharides, which have their origin in the amylolytic breakdown of the starch during the fermentation period and the early baking stage of the bread doughs. Evidence for the nature of these substances lies in the fact that the reducing non-fermentable substances obtained from loaves that do not contain lactose give glucose on hydrolysis with hydrochloric acid; and that treatment of the extract with takadiastase and subsequent fermentation greatly reduces the amount of, and in some cases completely eliminates, the reducing substances. The dextrinous nature of the substance explains the failure of Williams, Bevenue and Washaver[2] and of Munsey[3] to reduce or eliminate these substances by the use of ion-exchange resin.

The nature and the amount of these breakdown products of starch is influenced by, amongst other factors, the dough formula, the time and temperature of fermentation, the diastatic activity of the flour used, starch damage sustained during milling, water absorption of the flour and baking time and temperature. It becomes quite clear, therefore, that the amount and the nature of the reducing non-fermentable polysaccharides must differ from loaf to loaf, even if the same batch of flour has been used. When breads from an outside source are being analysed, the blank has to be computed from the results of analysis of a loaf baked from an entirely different flour from that used for the sample under test. It therefore becomes absolutely meaningless. Further, in this and other laboratories, blanks have been obtained occasionally that were larger than the combined amounts of lactose and reducing substance in the test sample.

It was decided consequently to develop a method that would permit the determination of lactose in bread without the necessity of carrying out a blank determination. Paper chromatography was used and was found to give satisfactory and reproducible results.

Experimental

As the determination of non-fat milk solids is generally carried out as a check on the claims of bakers about the milk-solids content of their bread, a method had to be found that would permit the chromatographic isolation of lactose within a reasonably short time. The only sugar occurring in bread that has a similar R_F value in most solvent mixtures to lactose is maltose, which is always present in varying quantities. The most suitable solvent mixture was found to be a mixture of *n*-butanol - pyridine - water (3 + 2 + 1·5), which forms a clear one-phase solution and gives excellent resolution in 48 hours by the descending

technique.[4] Very good resolution between lactose and maltose could be achieved within 24 hours by using a solvent mixture prepared by mixing three volumes of *n*-butanol - pyridine - water (3 + 2 + 1·5) mixture with one volume of acetone.

Direct photometry was used after the chromatogram had been dried and then sprayed with a 0·5 per cent. solution of 3:5-dinitrosalicylic acid in 4 per cent. sodium hydroxide solution and finally heated. With the available atomisers, however, sufficient uniformity could not be attained. Repeated spraying of the back and front of the chromatogram with a 0·1 per cent. solution of sodium 3:5-dinitrosalicylate, with drying after each application, produced satisfactory results, but was too tedious for a routine procedure.

The most convenient method was found to be the inclusion of silver nitrate in the solvent mixture and the heating of the chromatogram after drying and exposure to ammonia fumes. The inclusion of silver nitrate in the solvent necessitated the exclusion of acetone and, therefore, the time required for the resolution of lactose and maltose was doubled. Spraying with ammoniacal silver nitrate solution was not successful, as even prolonged drying and washing with ether did not remove the last traces of acetone from the paper.

Method

Reagents—

Ethanol, 80 per cent. v/v.
n-Butanol, b.p. 116° to 118° C.
Pyridine—Analytical-reagent grade.
Silver nitrate.
Ether.

Procedure—

Air-dry the sample and crush it. Cover 50 g of the sample with about 150 ml of ether and set the mixture aside for 3 hours with occasional shaking. Remove the ether by decanting, wash the sample with fresh ether on a Buchner funnel and then dry it.

Extract the sample with 250 ml of 80 per cent. v/v ethanol by heating under reflux for 2½ hours. After the mixture has cooled, filter on a Buchner funnel, wash the residue three times with 80 per cent. v/v ethanol and remove any liquid by pressing the residue. Discard the residue and transfer the clear filtrate to a 1-litre round-bottomed flask having a ground-glass joint, to which is fitted a Claisen distillation head; a capillary is placed in the flask. Remove the liquid by distillation under reduced pressure until only a small volume (about 10 ml) remains. Transfer the residue to a 50-ml calibrated flask. Wash the distillation flask and the capillary repeatedly with small amounts of hot water and add the washings to the contents of the calibrated flask. Allow the contents to cool, make up to volume with distilled water and mix thoroughly.

The solution is turbid and a sediment forms on cooling. The sediment is bulky and has a low solid content. Analysis of the dried sediment proved that it contained 65 per cent. of protein, probably gliadin, extracted from the bread by the ethanol. Washed gluten in the wet state contains between 65 to 70 per cent. of water. Despite partial denaturation of the extracted protein, its degree of hydration is very high and it has not been found necessary to make a correction for the volume it occupies. Keeping the flask in the refrigerator overnight causes complete settling of the solids and, after the contents of the flask have been allowed to attain room temperature, the clear brownish supernatant liquid can be spotted on to the filter-paper for chromatography.

Apply 2 μl of sample to a sheet of Whatman No. 1 filter-paper by means of a blood-pipette. In addition put 2-μl portions of lactose solutions, corresponding to 1, 2, 3, 4 and 5 per cent. of lactose in bread, on the same sheet. After drying the sheet, develop the chromatograms with the solvent mixture *n*-butanol - pyridine - water (3 + 2 + 1·5) containing 2·5 g of silver nitrate per 100 ml. To prevent darkening under the influence of light the chromatographic tank, if glass, must be suitably shielded.

After 48 hours, dry the chromatogram for 1 hour at room temperature in a fume chamber with the exhaust fan operating. Place it in a chromatographic tank from which the trough has been removed and into which a beaker containing concentrated ammonia solution has been placed, and set aside for 1 hour. Transfer the chromatogram without delay to an air-oven at 80° C ± 1° C and keep it there for 20 minutes. Then rinse the chromatogram with thiosulphate solution, wash it for 1 hour in running water and dry it.

Cut the chromatogram into strips and read the optical densities of the silver spots in a densitometer.

Results

In this laboratory a Spekker photo-electric absorptiometer H560, which has been converted for use as a densitometer, is in use.[5] A standard graph is prepared by plotting the log of the concentrations against the maximum optical densities. The concentration of the unknown sugar spots is then calculated from the standard graph.

When the log of the concentrations is plotted against the optical densities of the silver spots, a straight line is obtained. However, we have found it more convenient with our converted instrument to plot the concentrations on the abscissa, and the drum settings on the ordinate, when a straight line again results.

Analysis of the various non-fat dry milk solids used in this work carried out by the above method showed them to contain 50 per cent. of lactose. The optical densities of the silver spots from the lactose in the non-fat dry milk solids tested at levels corresponding to 1 to 5 per cent. of non-fat dry milk solids in bread (calculated on flour weight) also gave a straight line.

Breads containing various amounts of non-fat dry milk solids were baked and analysed by paper chromatography by spotting extracts from these breads on the same sheet of filter-paper in order of increasing milk content. The optical densities of the silver spots plotted against the non-fat dry milk contents of the breads gave a straight line, shown in Fig. 1.

Typical results for breads are given in Tables I and II.

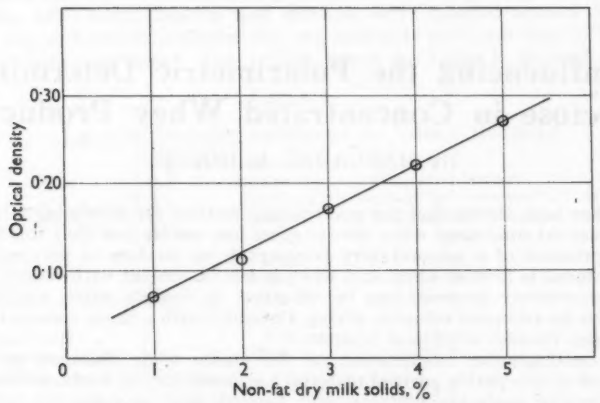

Fig. 1. Optical densities of the silver spots produced from bread extracts

Table I

Determination of non-fat dry milk solids in bread, carried out in triplicate by paper chromatography

Non-fat dry milk solids added (on flour weight), %	Non-fat dry milk solids found, %
1	0·9, 0·9, 1·1
2	1·9, 2·0, 2·0
3	3·1, 3·0, 3·0
4	3·9, 4·0, 4·0
5	5·0, 5·0, 4·9

Table II

Determination of non-fat dry milk solids in bread by paper chromatography

Non-fat milk solids added (on flour weight), %	1·0	1·5	2·0	2·5	3·0	3·25	3·5	3·75	4·0	4·25	4·5	4·75	5·0	6·0	9·0
Non-fat milk solids found, %	1·0	1·5	2·0	2·5	3·0	3·3	3·6	3·7	3·9	4·3	4·5	4·7	5·0	6·1	9·0

Discussion

The method outlined above eliminates the blank determination and thereby a potential source of error.

To obtain good results, the densities of the unknown must be within the range of the lactose standards. The standards must be run on the same chromatogram as the unknown, since the calibration graphs are not reproducible between chromatograms.

It has been found that with bread neither de-salting nor de-proteinising of the solutions is necessary. The spots formed on the chromatograms are well defined and no tailing takes place. If maltose standards are run on the same chromatogram, residual maltose in bread may be determined by this method.

I thank Miss M. L. Finlay for technical assistance and Mr. F. Hammer for baking the test loaves.

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Factors Influencing the Polarimetric Determination of Lactose in Concentrated Whey Products

By MARGARET A. HOUSE

It has been shown that the polarimetric method for determining lactose in commercial condensed whey always gives low results and that this is due to the presence of a laevorotatory polypeptide or mixture of polypeptides. This material is formed when acid whey is left in contact with rennet curd. The laevorotatory material can be adsorbed on Fuller's earth and eluted therefrom by ammonia solution, giving a product with a fairly constant ratio of nitrogen content to optical rotation.

Chromatographic examination of the amino acids obtained by acid hydrolysis of this partly purified material has shown that it contains: leucine, valine, proline, α-alanine, glutamic acid, aspartic acid, arginine and possibly lysine.

Whey, the major by-product of cheese-making, is chiefly of value as a source of lactose. This is prepared on a large scale by evaporating the whey *in vacuo* to give condensed whey, which contains about 60 per cent. of total solids and 45 per cent. of lactose. The sugar is allowed to crystallise and is then separated from the mother liquor and purified. An accurate knowledge of the amount of lactose in all the products obtained in this process is of great importance for economic manufacture. In the course of this work it has been found that the lactose content of condensed whey, and especially of the mother liquor, as determined from the optical rotation of a clarified serum, is always less than that determined by reduction of Fehling's solution.

It seemed possible that this effect could be due to the presence of reducing sugars other than lactose. Milk is known to contain minute traces of non-lactose sugars,[1,2] and ten oligosaccharides, in addition to glucose and galactose, have been detected as a result of hydrolysis of lactose in whey products by *Saccharomyces fragilis* lactase preparations.[3] However, this possibility was excluded by showing that condensed whey normally contains only traces of material capable of reducing Barfoed's solution, and also by paper-chromatographic examination of condensed whey, which revealed only negligible amounts of sugars other than lactose.[4]

Analytical methods

Before the problem was investigated further, various methods of preparing the necessary sera were examined. The methods finally used were those that, when applied to liquid milk, had been proved to give results in substantial agreement with the A.O.A.C. method for the determination of lactose in liquid milk.

Serum for polarimetric method—

Zinc acetate - potassium ferrocyanide precipitants were used as in the method recommended by the Milk Products Sub-Committee of the A.M.C. for the analysis of sweetened condensed milk.[5] All polarimetric readings were made at 20° C in a 2-dm tube, light from a sodium-vapour lamp being used.

Serum for Fehling's titration—

Saturated lead acetate solution was used as precipitant, the excess of lead being removed by adding solid potassium oxalate. The Fehling's titrations were carried out by the Lane and Eynon method.[6] During the course of the work, it was found that calculations from the published tables always gave slightly low results for pure lactose, and therefore a constant-volume technique in which the Fehling's solution was standardised against laboratory-recrystallised lactose has now been adopted for all such lactose determinations.[7]

Experimental

Analyses were carried out on a series of milk and whey products, and in addition the polarimetric sera, after neutralisation and dilution, were titrated against Fehling's solution to ensure that any differences between the two methods were not due to loss of lactose in preparing the polarimetric sera. The results, given in Table I, showed reasonably good

Table I

Lactose figures obtained by three methods

Product	Hydrated lactose by Fehling's method, %	Hydrated lactose by Fehling's method on polarimetric serum, %	Hydrated lactose by polarimeter, %
Separated milk	4·23	4·34	4·29
Evaporated milk..	11·51	11·59	11·53
Roller dried full-cream milk powder ..	34·5	34·5	34·9
Fresh whey	5·16	5·21	5·25
Commercial cheese whey	4·48	4·41	4·16
Commercial condensed whey	37·8	37·3	32·3
Commercial mother liquor from condensed whey	14·3	13·6	4·5
Wet crude lactose from condensed whey	64·3	64·6	63·4
Commercial dried mother liquor ..	27·5	25·2	Negative rotation
Mother liquor from condensed hydrochloric acid whey	—	15·5	15·5

agreement between the lactose determined by Fehling's method on the two types of sera, but the polarimetric results were low for commercial liquid and condensed whey, very low for the mother liquor separated from condensed whey and slightly low for wet crude lactose crystals, whereas with mother-liquor powder no results could be obtained, as the serum was laevorotatory. For all the other samples the results were in agreement, and it was therefore concluded that the anomalous results were due to the presence of a soluble laevorotatory substance that was not present in liquid milk and was not formed during heating, condensing or drying operations.

In order to discover when this laevorotatory substance was formed, samples of milk and whey were taken at various stages in the course of laboratory cheese manufacture. Table II shows results typical of those obtained on a number of different occasions; results obtained on two commercial samples have been included for comparison.

These results (Table II) show that the laevorotatory material was produced in the laboratory only when milk was soured by incubation with starter organisms, or when acid whey was kept in contact with cheese curd. Samples of milk and of whey after various periods in contact with curd at 39° C were then analysed to find out what other differences

TABLE II

LACTOSE CONTENT AT CONSECUTIVE STAGES IN CHEESE MAKING

Description of sample	Equivalent stage in factory cheese making	Acidity, % of lactic acid	Lactose by Fehling's method, %	Lactose by polarimeter, %
Separated milk	Whole milk	0·130	5·04	5·01
Separated milk inoculated with cheese starter and held overnight at 72° F	Bulk starter	0·820	4·27	4·07
Separated milk with 1 per cent. of starter, after standing for 1 hour at 86° F	Milk just before addition of rennet	0·160	5·03	5·03
Whey sampled ¾ hour after addition of rennet	Cutting	0·090	5·30	5·35
Most of the whey drained after a further hour, temperature 86° to 98° F	Whey off	0·120	5·26	5·32
Remainder of whey left with curd overnight and then drained	Whey at pressing	0·830	4·11	3·61
Commercial samples	Whey as drawn off	0·430	4·22	4·13
	Whey at pressing	0·550	2·18	2·03

TABLE III

COMPOSITION OF FRESH AND STORED WHEY

	Original milk	Fresh whey	Whey kept with curd for 1 day	2 days	3 days
Sample 1—					
Total solids, %	—	6·50	6·48	6·70	6·83
Protein (N × 6·38), %	—	0·96	0·95	1·10	1·23
Acidity, % of lactic acid	0·135	0·130	0·475	0·620	0·695
Lactose by Fehling's method, %	4·84	4·97	4·53	4·23	4·18
Lactose by polarimeter, %	4·85	5·00	4·30	4·02	3·78
Sample 2—					
Total solids, %	9·64	7·00	7·25	—	—
Protein (N × 6·38), %	3·60	0·98	1·08	—	—
Acidity, % of lactic acid	0·15	0·11	0·68	—	—
Lactose by Fehling's method, %	5·11	5·38	4·71	—	—
Lactose by polarimeter, %	5·19	5·43	4·37	—	—
Ash, %	0·80	0·57	0·76	—	—

occur when the laevorotatory substance is formed. The results are given in Table III. It was always found that the formation of the laevorotatory substance was accompanied by increases in the ash, acidity and "protein" contents of the whey.

Different fractions of the whey were removed consecutively by the following methods—

(*a*) boiling and filtering through Filtercel or kieselguhr; this removed the heat-coagulable protein,

(*b*) stirring with, or passing through a column of, ion-exchange resin MB1; this removed the cations and anions, and

(*c*) stirring with Fuller's earth to remove some of the non-coagulable nitrogen fraction.[8]

The original whey and the various filtrates were analysed, and the results are shown in Table IV. From these results it appeared that neither the protein removed by boiling and filtering, nor the ash, nor the acidity, had any effect upon the rotation, but Fuller's earth and ion-exchange resins removed the laevorotatory substance together with part of the non-coagulable nitrogenous material.

PREPARATION AND ISOLATION OF LAEVOROTATORY MATERIAL—

In a further series of experiments the optimum conditions for the formation of the laevorotatory substance were found to be incubation of rennet curd with 1·6 per cent. lactic acid solution at 39° C. Presence of starter organisms was not necessary. If the rennet curd was washed free from lactose before being suspended in the lactic acid solution, the

resultant serum had a negative rotation. Curd precipitated by acid, without the use of rennet, and then treated in the same manner gave a strongly laevorotatory clear filtrate, but when a polarimetric serum was prepared from this, it was optically inactive. Hence two types of laevorotatory material are obtainable from curd. One is formed by the action of acid alone, and is removed by the zinc ferrocyanide precipitants, whereas the other, which causes anomalies in the analysis of commercial cheese whey products, requires the united action of rennet and acid, and is not precipitable. The first of these materials may also be present in commercial whey products, but it cannot be detected because of the necessity of preparing a clear serum for polarimeter readings.

TABLE IV

COMPOSITION OF WHEY AFTER VARIOUS TREATMENTS

	(1) Original whey	(2) (1) boiled and filtered through Filtercel	(3) (2) stirred with Fuller's earth	(4) (3) stirred with ion-exchange resin MB1	(5) (1) boiled and filtered through kieselguhr	(5) stirred with ion-exchange resin MB1
Total solids, % ..	7·21	6·83	6·47	4·56	—	—
Protein, %	1·07	0·69	0·25	0·25	0·73	0·52
Ash, %	0·79	0·81	0·89	0·02	0·80	0·01
Acidity, % of lactic acid	0·70	0·66	0·44	0·05	—	—
Lactose by Fehling's method, % ..	4·61	4·58	4·58	4·53	4·72	4·59
Lactose by polarimeter, %	4·40	4·29	4·56	4·61	4·51	4·61

Under these optimum conditions, several batches of whey were prepared that showed large discrepancies between the lactose determined by Fehling's method and that by polarimeter. The wheys were condensed and the mother liquors were separated. Sera were prepared by the addition of trichloroacetic acid, the excess of precipitant being removed by extraction with ether,[9] and measured volumes were stirred with Fuller's earth that had previously been extracted with 4 per cent. ammonia solution, washed with water and dried. After the mixture had been stirred for approximately 1 hour, the Fuller's earth was filtered off and washed several times with water, eluted by repeated stirring with fresh quantities of 4 per cent. ammonia solution and finally washed free from ammonia with distilled water. The ammoniacal extracts and washings were combined and evaporated to a small volume under vacuum. The last traces of ammonia were removed by adding 0·1 *N* barium hydroxide and further evaporating; the barium was removed as barium sulphate. The final solution was made up to the volume of the trichloroacetic acid serum that had been treated with Fuller's earth. Analyses of the trichloroacetic acid sera before and after treatment with Fuller's earth, and of the final material eluted from the Fuller's earth, showed that this material was of a similar composition to the portion that had been removed from the original sera. Recovery of the adsorbed material was, however, incomplete. In six experiments the rotation equivalent to 1 per cent. of nitrogen in the extracts varied between −13·1° and −15° C. Similar results were obtained with a lactic acid solution incubated with a washed rennet curd.

Some of these partly purified eluates were analysed chromatographically. One-dimensional paper chromatograms run in *n*-butanol - acetic acid - water and developed with ninhydrin showed considerable tailing, suggesting that the material contained a mixture of polypeptides. Two-dimensional chromatograms run in *n*-butanol - acetic acid - water followed by phenol - water confirmed this. The extracts were therefore hydrolysed by heating under reflux with hydrochloric acid, and the amino acids in the hydrolysates were identified by one-dimensional paper chromatography, *n*-butanol - acetic acid - water being used as solvent. The chromatograms showed the presence of leucine, valine, proline, α-alanine, glutamic acid, aspartic acid, arginine and possibly lysine.

To confirm that the Fuller's earth adsorbed from whey that portion causing the original discrepancy, a bulk of boiled and filtered whey showing the effect was divided into two portions, the first being concentrated without further treatment and the second after treatment with Fuller's earth. The mother liquors were separated and their lactose contents were

determined by both methods. The results are shown in Table V, and they confirm that the Fuller's earth removed the substance responsible for the anomalous results.

TABLE V

LACTOSE IN MOTHER LIQUORS

	Lactose by Fehling's method, %	Lactose by polarimeter, %
Mother liquor from control whey	13·4	5·2
Mother liquor from whey treated with Fuller's earth ..	16·9	16·6

DISCUSSION

During the course of the work it has been thought that the discrepancy observed between the Fehling's and polarimetric values for lactose could be due to the laevorotatory products of one or more of the following four reactions: (*a*) proteolysis due to the growth of starter organisms, (*b*) hydrolysis of lactose due to the growth of starter organisms, (*c*) action of lactic acid on acid-precipitated casein, and (*d*) action of lactic acid on casein after coagulation by rennet.

Considering these possibilities in order—

(*a*) Proteolysis in cultures of *Streptococcus lactis* causing rapid increase in soluble nitrogen has been reported.[10,11] There is no indication in these papers of any change in optical activity, but it seems a likely corollary, and therefore this reaction probably accounts for the results of the analyses of bulk cheese starters, *e.g.*, the sample quoted in Table II. It could only account for a minute portion of the discrepancy between the Fehling's and polarimetric values for lactose in condensed whey, because of the small amount of such starter used, *i.e.*, $\frac{1}{2}$ to 1 per cent. It is possible that the starter organisms continue to hydrolyse the casein while the milk is in the vat and may subsequently hydrolyse the whey proteins, but experimental results have shown that the optically active products of such proteolysis are not adsorbed by Fuller's earth and therefore cannot be identical with the material obtained from condensed whey.

(*b*) Some cultured milks have been found to contain sugars that reduce Barfoed's reagent,[12] and this could account for some anomalies in the analysis of bulk starters, but not for the effect observed in condensed whey, since no such sugars were found in samples that showed large discrepancies.

(*c*) Acid-precipitated curd washed free from lactose and then suspended in lactic acid solution gave rise to a laevorotatory material, but this was removed by the precipitants used in making the polarimetric sera and therefore it could not account for the results found with condensed whey.

(*d*) The reaction between lactic acid and rennet curd made without the addition of starter organisms has been shown to give rise to a laevorotatory polypeptide or mixture of polypeptides similar in properties to that found in condensed cheese whey. These polypeptides can be adsorbed on Fuller's earth and the eluates therefrom account for nearly all the discrepancies in the original analyses.

In a series of partly purified preparations of this laevorotatory material, the relation between nitrogen content and optical rotation was not constant, but the variations were small, considering the large number of operations necessary to prepare the concentrated materials. It therefore seems justifiable to assume that this polypeptide mixture is formed by a particular reaction that takes place during the cheese-making process and appears to be acid hydrolysis of one of the products of rennet action. In the laboratory it was possible to prepare fresh rennet whey showing no discrepancy, but all the factory wheys and commercial condensed wheys examined gave lower results for lactose by the polarimetric method than by Fehling's method. In the cheese factories the curd is not separated as completely from the whey as is possible in the laboratory and so the conditions necessary for the production of the laevorotatory material are always present.

I thank Dr. M. M. Muers for his advice and encouragement.

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Recommended Methods for the Analysis of Trade Effluents

PREPARED BY THE JOINT A.B.C.M. - S.A.C. COMMITTEE ON METHODS FOR THE ANALYSIS OF TRADE EFFLUENTS

Sampling and Physical Examination of the Sample

Sampling

General considerations

Information to be recorded at the time of sampling should include—

details of type of sample,
the place and time of sampling,
the temperature of sample, and
any other relevant data.

Industrial effluents often vary so rapidly and so widely in character that "snap" samples may not afford a reliable guide to the nature of the over-all discharge. In such instances a suitable number of individual samples should be taken at appropriate intervals and made into a composite sample. When practicable, the volumes of the individual samples should be related to the volume of effluent flowing at the time.

In certain circumstances it may be more convenient for a composite sample to be prepared by the analyst, who, for this purpose, will require a record of the approximate flow of effluent at the time each individual sample was taken.

Some effluents are of such a heterogeneous character that general directions for sampling cannot be given. The precise technique to be adopted in such instances should be agreed between the analyst and the other parties concerned.

Although the amount of sample to be taken should be agreed with the analyst, at least 2 litres will usually be required for a complete range of tests. The sample should be contained in a clean glass bottle with a ground stopper. Wide-mouthed bottles kept specially for the purpose are required when sampling oily liquids. New bottles should be washed with acid and thoroughly rinsed with distilled water before being brought into use.

For certain tests special samples will be required, and these should be taken as described later.

Special considerations

Unstable effluents—

When effluents contain dissolved gases, readily oxidisable substances or other substances that may disappear during transit, special precautions may be necessary, *e.g.*, samples intended for the determination of dissolved oxygen should be taken with an under-water sampling device such as the Casella apparatus,* or the apparatus described in "Standard Methods for the Examination of Water, Sewage and Industrial Wastes," Tenth Edition (p. 251), published by the American Public Health Association Inc.; samples in which traces of sulphide are to be determined should be analysed without delay or treated immediately with a suitable fixative, *e.g.*, zinc acetate.

Effluents containing immiscible liquids—

The proportionate ratio of two immiscible liquids of different specific gravities (*e.g.*, mineral oil and water) usually cannot be maintained in a sample obtained by dipping a jug or other container into an effluent that is flowing in or from a pipe. The most satisfactory method of sampling two-phase liquids is to use a sampling tube that is capable of withdrawing a complete section of the effluent as it flows in a rectangular culvert or trough: in most instances, however, effluents will have

* Obtainable from Messrs. C. F. Casella & Co. Ltd., Regent House, Fitzroy Square, London, W.1.

to be sampled from the outfall of a pipe or from a stream and in these circumstances some of the effluent should first be collected in a large cylindrical vessel having a capacity of two to three gallons. A sectional sampling tube should be used to

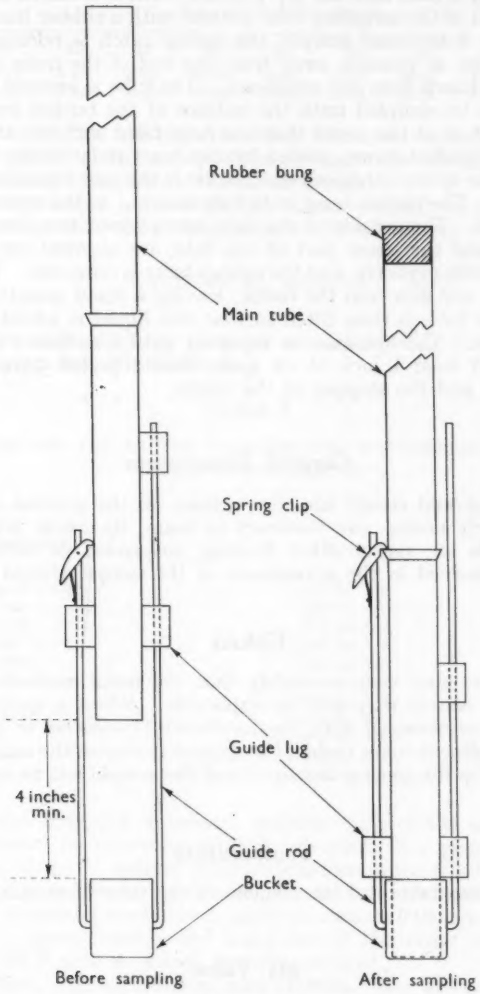

Fig. 1. Sampling tube

withdraw the test sample from this. A sampling tube suitable for sampling effluents that do not contain highly viscous matter (*e.g.*, tar) is described below (see Fig. 1); the measurements are approximate.

The sampler consists of a heavy-gauge brass tube, 3 feet long, with an outside diameter of $1\frac{1}{2}$ inches. Over one end of the tube is fitted a brass bucket made from a piece of tube 2 inches long and sealed at one end. The bucket has an internal diameter $\frac{1}{16}$ inch greater than the outside diameter of the main tube. To opposite sides of the bucket are brazed two brass rods, $\frac{1}{4}$ inch in diameter, which pass through guides brazed to the sides of the main tube. The rods are so arranged that the top of the bucket can be withdrawn to a distance of not

less than 4 inches from the bottom of the main tube, and they guide the bucket into a position covering the end of the tube when it is pushed back again. A suitable spring catch is provided on one of the guide rods so that the bucket is automatically locked into the top position when it is raised to its highest point. The open end of the sampling tube is fitted with a rubber bung.

To take a sectional sample, the spring catch is released and the bucket is drawn as far as possible away from the end of the main tube. The rubber bung is withdrawn from the other end. The tube is lowered vertically through the liquid to be sampled until the bottom of the bucket rests on the bottom of the culvert or of the vessel that has been filled with the effluent. The main tube is then pushed down, guided by the brass rods, to the limit of its travel, whereupon the spring catch locks the bucket in the raised position covering the end of the tube. The rubber bung is tightly inserted in the open end and the tube is withdrawn. The outside of the sampler is wiped free from adhering liquid, the bucket and the lower part of the tube are inserted into a wide-mouthed bottle of suitable capacity, and the rubber bung is removed. The sample section of the liquid will flow into the bottle, leaving a small quantity of liquid in the bucket. The tube is then tilted so that this liquid is added to the main bulk of the sample. The operation is repeated until a sufficient quantity has been collected. At least 1 inch of air space should be left between the top level of the liquid and the stopper of the bottle.

General Description

The analyst should record his observations on the general appearance of the sample, particularly noting any tendency to foam, its colour and odour, and the presence of visible oil, tar or other floating, suspended or settled matter. Any change that is observed in the appearance of the sample should be recorded.

Colour

Trade effluents may vary so widely that the usual methods for determining colour in potable waters may not be applicable. When a quantitative figure is required, it may be obtained with the Lovibond Tintometer or other comparable instrument if the effluent is not turbid, but in most instances the analyst's observation of colour included in the general description of the sample will be sufficient.

Temperature

Record the temperature of the effluent at the time of sampling to the nearest 0·5° C.

pH Value

The pH value of an effluent may be determined either electrometrically or colorimetrically. The colorimetric method is convenient and sufficiently accurate for general work when the sample is relatively free from colour and turbidity.

Electrometric method

Apparatus—

Any reliable commercial instrument with a glass electrode may be used; it should be standardised by one or both of the buffer solutions specified below.

Standard buffer solution of pH 4—Dissolve 10·21 g of anhydrous potassium hydrogen phthalate, $COOH{\cdot}C_6H_4{\cdot}COOK$, in boiled and cooled distilled water and dilute the solution to 1 litre.

Standard buffer solution of pH 9—Dissolve 19·07 g of borax, $Na_2B_4O_7.10H_2O$, in boiled and cooled distilled water and dilute the solution to 1 litre.

The pH of these buffer solutions varies with temperature in accordance with the following table—

	True pH at temperature, ° C, of									
Buffer of	5	10	15	20	25	30	35	40	45	50
pH 4 ..	4·01	4·00	4·00	4·00	4·01	4·01	4·02	4·03	4·04	4·06
pH 9 ..	9·38	9·33	9·27	9·22	9·18	9·14	9·10	9·07	9·04	9·01

It should be noted that the glass-electrode instrument cannot be used directly on oily or greasy effluents, but an approximate value for the pH can be obtained if these are first filtered to remove the oil or grease.

Special glass electrodes are necessary for alkaline liquids of pH greater than 9. The precautions recommended by the makers of the instrument should be observed.

Colorimetric method

Reagents—

The indicators most generally used for the colorimetric determination of pH values of effluents are listed in Table I.

Table I

Indicators for use in the colorimetric determination of pH values of effluents

Indicator	pH range	Volume of 0·1 *N* sodium hydroxide required per 0·1 g of indicator, ml
Thymol blue (acid range)	1·2 to 2·8	2·15
Bromophenol blue	2·8 to 4·6	1·5
Bromocresol green	3·6 to 5·2	1·45
Chlorophenol red	4·8 to 6·4	3·15
Bromothymol blue	6·0 to 7·6	1·6
Phenol red	6·8 to 8·4	2·85
Cresol red	7·2 to 8·8	2·65
Thymol blue (alkali range)	8·0 to 9·6	2·15
Phenolphthalein	8·2 to 10·0	—
Thymolphthalein	9·3 to 10·5	—
Thymol violet	9·0 to 12·0	—

To prepare the indicator solutions, moisten 0·1 g of the solid indicator with about 2 ml of ethanol (or industrial methylated spirit) in a glass mortar, triturate with the amount of 0·1 *N* sodium hydroxide shown in the third column of Table I and dilute with distilled water to 25 ml. Dilute this stock solution with 20 per cent. ethanol (or industrial methylated spirit) to make a 0·04 per cent. solution and adjust the pH to approximately the mid-point of the range by the addition of approximately 0·02 *N* acid or alkali, freshly prepared.

Phenolphthalein, thymolphthalein and thymol violet are used in 0·1 per cent. solution in 95 per cent. ethanol (or industrial methylated spirit) and any acidity in the alcohol should be neutralised with alkali after the indicator solution has been prepared.

All indicators should be stored in resistance-glass containers with ground stoppers; corks should not be used.

Procedure—

First determine the approximate pH value of the sample by means of a universal indicator and then select the appropriate indicator, avoiding extremes of the range. Transfer by pipette 0·4 ml of the indicator solution into a clean test-tube of colourless glass, kept specially for the purpose, and add from a pipette 10 ml of the sample (avoid agitation), mixing gently with the pipette. Compare with buffer

tubes of different pH values prepared with this indicator. Directions for the preparation of buffer solutions covering the range pH 1·5 to 11 are given in "Thorpe's Dictionary of Applied Chemistry," Fourth Edition, Volume 2, p. 122, and in other standard works.

If the sample is coloured, compensate for its colour by using a comparator in which an extra tube of 10 ml of the sample, without indicator, can be viewed with the buffer tube.

NOTES—1. Alternatively, commercial instruments that are available for the colorimetric determination of pH may be used, provided that the maker's instructions are followed.

2. In water containing residual free chlorine, the colour of the indicator may be affected and the colorimetric method is not applicable.

Transparency

PRINCIPLE OF METHOD—

The depth of liquid at which a standard mark is just obscured is taken to be a measure of the transparency.

APPARATUS—

A colourless glass tube, 620 ± 10 mm long and of internal diameter 25 ± 1 mm, fitted with a plane glass bottom and graduated in millimetres from the bottom to a height of 50 mm and at every 5 mm from 50 mm to 600 mm. This tube is fitted with a side tube, 6 to 7 mm in diameter, at a height of about 50 mm from the bottom, connected by rubber tubing to a glass reservoir, having a capacity of 300 ml. A black cross with lines 1 mm wide on white paper is pasted on the outside of the bottom of the glass tube so that the cross can be observed down the tube.

PROCEDURE—

Support the tube vertically, preferably illuminated by north daylight. Pour in the liquid slowly, observing the depth of liquid at which the black cross just disappears. If this depth is greater than the height of the side arm, variation of depth can most easily be attained by suitable manipulation of the reservoir.

Report the transparency as the depth of the column in millimetres through which the cross is just not visible.

NOTES—1. Observations may be made on the sample after a suitable period of settlement or on the sample when uniformly mixed.

2. The determination of transparency cannot be made with great precision; it should be noted that the transparency may vary considerably with the time that has elapsed since the sample was taken.

Settleable Solids

The amount of settleable solids is defined as the weight in milligrams per litre of insoluble matter that will settle from an effluent under prescribed conditions. It is often convenient to specify a period of 4 hours for settlement, but the appropriate time will vary with the purpose for which the information is desired, and it must be left to the discretion of the analyst, who should report the settling time adopted.

PROCEDURE—

Fill a 100-ml Nessler cylinder to the upper mark with the well mixed sample. Allow the sample to stand undisturbed at a temperature between 10° and 15° C for the specified time. Without disturbing the sediment, withdraw the top 50 ml and determine the amount of suspended solids in the separated liquid by the method prescribed for "Total Suspended Solids." Calculate the results in milligrams per litre and deduct this figure from that found for total suspended solids. The difference between the two values is recorded as "settleable solids."

Total Suspended Solids

Samples containing an excessive amount of suspended matter, and those containing colloidal matter, are often difficult to filter. When it is impracticable to use the filtration method, the centrifugal method should be used.

NOTE—If the suspended matter includes volatile oils, the following procedures only measure the non-volatile part. The centrifugal method is not applicable if any part of the suspended matter floats.

FILTRATION METHOD

PRINCIPLE OF METHOD—

Suspended matter is determined by weighing the residue after filtering the effluent through an asbestos pad in a Gooch crucible.

REAGENT—

Asbestos cream—Prepare a cream with distilled water by adding 15 g of acid-washed medium-fibre asbestos (prepared specially for use with Gooch crucibles) to 1 litre of distilled water. Some asbestos supplied for this purpose contains too much fine asbestos powder; this fine material should be removed by repeated decantations.

PROCEDURE—

Prepare an asbestos fibre pad in a 30-ml Gooch crucible by adding sufficient of the asbestos cream to produce a pad 3 mm thick and containing about 0·3 g dry weight of asbestos. Wash the pad with distilled water, dry the whole at 100° to 105° C for 1 hour, cool and weigh. If mineral suspended solids are subsequently to be determined, the crucible and pad must first be ignited at 500° C and cooled before weighing.

It is desirable to use the maximum volume of the well mixed sample that can be passed through the crucible without clogging the filter-pad. Filter successive increments of 10 ml of the well shaken sample, using gentle suction, and add each increment just before the pad becomes dry; continue until the rate of filtration becomes inconveniently slow, or until about 200 ml of the sample have been used. If a pipette is used for measuring the increments, the orifice should be wide enough to prevent it becoming clogged during the operation. Carefully wash the pad with 5 ml of distilled water, dry the whole at 100° to 105° C and weigh. Express the results as milligrams of suspended solids per litre of sample.

If a measurement of the dissolved solids is required, retain the filtrate.

If a determination of the mineral suspended solids is required, ignite the crucible in a muffle furnace at a temperature not exceeding 500° C for 15 minutes, cool and re-weigh.

CENTRIFUGAL METHOD

APPARATUS—

Vol. 81, 1956: August, p. 497.

Replace the last 4 lines of text and the first 2 lines of the footnote by—

usual way and centrifuge the liquid for not less than 5 minutes at a relative centrifugal force of 1400 to 3000.* Decant the supernatant liquid, refill the tube with distilled water to the mark and centrifuge again for a further period of 5 minutes. Again decant the supernatant liquid and transfer the sediment with the aid of a wash-bottle to a weighed silica, porcelain or platinum dish. Dry the residue at 100°

* The relative centrifugal force = $1{\cdot}12 \times 10^{-5} \times rN^2$,

where r = the radius in centimetres from the centre of the head of the centrifuge to the closed end (bottom) of the tube when in rotation, and

N = the number of revolutions per minute.

Total Suspended Solids

Samples containing an excessive amount of suspended matter, and those containing colloidal matter, are often difficult to filter. When it is impracticable to use the filtration method, the centrifugal method should be used.

NOTE—If the suspended matter includes volatile oils, the following procedures only measure the non-volatile part. The centrifugal method is not applicable if any part of the suspended matter floats.

FILTRATION METHOD

PRINCIPLE OF METHOD—

Suspended matter is determined by weighing the residue after filtering the effluent through an asbestos pad in a Gooch crucible.

REAGENT—

Asbestos cream—Prepare a cream with distilled water by adding 15 g of acid-washed medium-fibre asbestos (prepared specially for use with Gooch crucibles) to 1 litre of distilled water. Some asbestos supplied for this purpose contains too much fine asbestos powder; this fine material should be removed by repeated decantations.

PROCEDURE—

Prepare an asbestos fibre pad in a 30-ml Gooch crucible by adding sufficient of the asbestos cream to produce a pad 3 mm thick and containing about 0·3 g dry weight of asbestos. Wash the pad with distilled water, dry the whole at 100° to 105° C for 1 hour, cool and weigh. If mineral suspended solids are subsequently to be determined, the crucible and pad must first be ignited at 500° C and cooled before weighing.

It is desirable to use the maximum volume of the well mixed sample that can be passed through the crucible without clogging the filter-pad. Filter successive increments of 10 ml of the well shaken sample, using gentle suction, and add each increment just before the pad becomes dry; continue until the rate of filtration becomes inconveniently slow, or until about 200 ml of the sample have been used. If a pipette is used for measuring the increments, the orifice should be wide enough to prevent it becoming clogged during the operation. Carefully wash the pad with 5 ml of distilled water, dry the whole at 100° to 105° C and weigh. Express the results as milligrams of suspended solids per litre of sample.

If a measurement of the dissolved solids is required, retain the filtrate.

If a determination of the mineral suspended solids is required, ignite the crucible in a muffle furnace at a temperature not exceeding 500° C for 15 minutes, cool and re-weigh.

CENTRIFUGAL METHOD

APPARATUS—

Centrifuge.

Cone-shaped centrifuge tubes, having capacities of 50 ml.

PROCEDURE—

Transfer 50 ml of the well mixed sample to a centrifuge tube, balance it in the usual way and centrifuge the liquid for not less than 5 minutes at 1400 to 3000 ***g***.* Decant the supernatant liquid, refill the tube with distilled water to the mark and centrifuge again for a further period of 5 minutes. Again decant the supernatant liquid and transfer the sediment with the aid of a wash-bottle to a weighed silica,

* ***g*** = the relative centrifugal force

$= 1{\cdot}12 \times 10^5 \times rN^2$,

where r = the radius in centimetres from the centre of the head of the centrifuge to the closed end (bottom) of the tube when in rotation, and

N = the number of revolutions per minute.

porcelain or platinum dish. Dry the residue at 100° to 105° C for 1 hour and weigh
Express the result as milligrams of suspended solids per litre of sample.

If a measurement of the dissolved solids is required, retain the clear dec
liquid and washings.

If desired, the residue can be ignited, as in the filtration method, to deter
the mineral suspended matter.

Residue on Evaporation

The residue on evaporation is the total solid matter obtained under the prescribed conditions.

NOTE—In the presence of substantial quantities of hygroscopic substances, *e.g.*, calcium chloride, an accurate result cannot be obtained by this method.

PROCEDURE—

Evaporate on a water bath 100 ml of the well mixed sample in a previously heated and weighed basin. Dry the residue at 100° to 105° C in an oven for 1 hour, cover the basin with a clock-glass, cool in a desiccator and reweigh the basin. Express the result as milligrams of residue per litre of sample.

Dissolved Solids

The dissolved solids may be calculated indirectly as the difference between the residue on evaporation and the total suspended solids. However, if the dissolved-solids content in the effluent is low and the suspended-solids content is high, a direct determination is necessary. It is preferable to adopt the centrifugal method of separating suspended matter in order that a sufficiently large volume of separated liquid shall be available for this determination.

PROCEDURE—

Transfer quantitatively to a previously weighed basin the clear liquid from which the suspended solids have been separated by filtering or centrifuging. Evaporate it to dryness on a water bath. Dry the residue at 100° to 105° C in an oven for 1 hour, cover the basin with a clock-glass, cool in a desiccator and reweigh the basin. Express the result as milligrams of dissolved solids per litre of sample.

Vol. 81, 1956: August, p. 498.

Replace the first 2 lines by—

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PROCEDURE—

Evaporate on a water bath 100 ml of the well mixed sample in a previously heated and weighed basin. Dry the residue at 100° to 105° C in an oven for 1 hour, cover the basin with a clock-glass, cool in a desiccator and reweigh the basin. Express the result as milligrams of residue per litre of sample.

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Transfer quantitatively to a previously weighed basin the clear liquid from which the suspended solids have been separated by filtering or centrifuging. Evaporate it to dryness on a water bath. Dry the residue at 100° to 105° C in an oven for 1 hour, cover the basin with a clock-glass, cool in a desiccator and reweigh the basin. Express the result as milligrams of dissolved solids per litre of sample.

Notes

A COLOUR REAGENT FOR CATIONS

MANY reagents have been suggested for the detection of cations in paper-chromatographic experiments and this Note describes the use of a rubeanic acid - pentacyanide complex for this purpose.

Rubeanic acid has frequently been employed for the detection of copper, nickel, cobalt, bismuth, manganese, lead, cadmium, mercury, iron and silver.[1] The use of potassium ferrocyanide has been suggested for the detection of iron, molybdenum, copper, the uranyl radical, niobium, titanium, cobalt, vanadium and tungsten.[1] The reagent described below, which is made from rubeanic acid and trisodium pentacyanoamminoferrate, gives characteristically coloured salts insoluble in acetic acid with most of the elements listed above and in addition gives colours with zinc, tin, titanium, zirconium, hafnium, thorium, palladium and gold.

The complex anion used in this test does not appear to have been described previously in any detail, although it is probably the same complex as that present in the violet solution prepared by the reaction of rubeanic acid with trisodium pentacyanoaquoferrate.[2]

EXPERIMENTAL

Trisodium pentacyanoamminoferrate is prepared by the reaction of ammonia with sodium nitroprusside.[3] The reagent is prepared by dissolving 0·70 g of trisodium pentacyanoamminoferrate, $Na_3[Fe(CN)_5NH_3].6H_2O$, in 20 ml of water and by pouring the resulting solution into a solution of 0·25 g of rubeanic acid in 10 ml of ethanol. The mixture, which is then shaken for 15 minutes, is ready for use after filtration. If the solution is kept at room temperature, *i.e.*, at approximately 20° C, it should be made on the day it is to be used, but if it is kept at — 15° C it can be stored for at least 1 month. Approximately 0·2 *N* acetic acid is prepared by diluting 11 ml of glacial acetic acid to 1 litre with water.

The colours produced by various salts were studied by placing a drop of solution (0·05 ml) containing the metal salt on a filter-paper, which was then sprayed with the reagent and washed with 0·2 *N* acetic acid until the colour had been removed from that part of the paper not impregnated with the metallic salt. The metal-salt solutions were neutral or faintly acid before spraying. The reagent itself is violet, but becomes brown in the presence of the acetic acid. All salt solutions employed for the test were 0·1 *M*, except for the following: auric chloride, 10 g of metal per litre; hafnium, 5 g of HfO_2 per litre dissolved in hydrochloric acid; niobium, 0·06 g of Nb_2O_5 per litre dissolved in ammonium oxalate; tantalum, 0·4 g of Ta_2O_3 per litre in ammonium oxalate; rhodium, palladium, iridium and platinum, 1 g of metal per litre; the lanthanum, praseodymium, neodymium, samarium, gadolinium and holmium solutions were 0·01 *M*. The colours obtained in the experiment are listed in Table I. Some of the differences in the colours observed for metals in the same periodic group, *e.g.*, Zn^{++} brick red and Cd^{++} blue, and for metals in different valency states, *e.g.*, Hg^{++} green and Hg^{+} brown, are remarkable. Several of the colours are intense, and as an example of the use of this reagent in paper-chromatographic experiments the separation of Ni^{++}, Mn^{++}, Co^{++} and Zn^{++} may be quoted. A drop of solution containing 60 μg of each of these metals was placed on a strip of Whatman No. 1 filter-paper, which was then dried. A solvent prepared from 5 parts of water, 8 parts of concentrated hydrochloric acid and 87 parts of acetone was allowed to flow down the paper.[4] The paper was removed and air-dried when the solvent front had moved 25 cm. The paper was suspended over concentrated ammonia solution for a few minutes and again hung in the air. The paper was sprayed with the rubeanic acid - pentacyanide reagent and was washed with 0·2 *N* acetic acid. The following bands were observed, reading from the top: Ni^{++} blue, Mn^{++} blue, Co^{++} brown, Zn^{++} red and Fe^{+++} green. The Fe^{+++} band was in the solvent front and was due to impurity. The various blue bands were of different hue.

The colours do not appear to fade appreciably on keeping but do show some change of shade. For example, the lead, zirconium, thorium and nickel spots turned blue grey, the bismuth spot turned green grey and the mercurous spot turned green.

The relative sensitivities of the proposed reagent and of rubeanic acid were compared by placing 0·02 ml of the appropriate solution containing the metal ion (0·1, 0·01, 0·001 or 0·0001 *M*) on Whatman No. 1 filter-paper and by spraying the dried paper with the selected reagent. The papers treated with the new reagent were washed with 0·2 *N* acetic acid and those treated with 0·5 per cent. of rubeanic acid in ethanol were hung over ammonia solution, sp.gr. 0·880. In this way it was shown that the new reagent has a sensitivity greater than that of rubeanic acid for the detection of Au^{+++}, Zn^{++}, Zr^{++++}, Hf^{++++} and Th^{++++}, a sensitivity equal to that of rubeanic acid

for the detection of Cu^{++}, Ag^{+}, Cd^{++}, Ti^{+++}, Bi^{+++}, Mn^{++}, Fe^{+++}, Co^{++} and Pd^{++} and a sensitivity less than that of rubeanic acid for the detection of Hg^{+}, Hg^{++}, Sn^{++}, Sn^{++++}, Pb^{++} and Ni^{++}. Under the conditions described 1 μg of the following metal ions could be detected by the new reagent: Cu^{++}, Zn^{++}, Hf^{++++}, Th^{++++}, Fe^{+++}, Co^{++}, Ni^{++} and Pd^{++}. Sharper spots were observed with the new reagent than with rubeanic acid when the 0·1 *M* solutions of Fe^{+++}, Co^{++} and Mn^{++} were employed, and the white background obtained in all the experiments was found to be very convenient for the photo-electric measurement of the intensities of the spots.

TABLE I

SPOTS ON FILTER-PAPER OBSERVED AFTER SPRAYING WITH REAGENT AND WASHING WITH 0·2 *N* ACETIC ACID

Elements arranged in periodic groups

Group IA:	Na^{+}, no colour
Group IB:	Cu^{++}, brown black; Ag^{+}, greyish brown; Au^{+++}, green
Group IIA:	Mg^{++}, no colour; Ca^{++}, no colour
Group IIB:	Zn^{++}, brick red; Cd^{++}, blue; Hg^{++}, green grey; Hg^{+}, greyish brown
Group IIIA:	Al^{+++}, no colour; Tl^{+}, pale grey; the rare earths La^{+++}, Ce^{+++}, Pr^{+++}, Nd^{+++}, Sm^{+++}, Gd^{++} and Ho^{+++}, very pale blue
Group IVA:	Sn^{++++}, pale red brown; Sn^{++}, red brown; Pb^{++}, purplish grey
Group IVB:	Ti^{+++}, brown; Zr^{++++}, red purple; Hf^{++++}, red purple; Th^{++++}, red purple
Group VA:	Sb^{+++}, no colour; Bi^{+++}, brown
Group VB:	VO_4''', no colour; Nb, no colour; Ta, no colour
Group VIB:	Cr^{+++}, no colour; molybdate, very pale brown; WO_4'', no colour; UO_2^{++}, brown
Group VIIB:	Mn^{++}, blue
Group VIII:	Fe^{+++}, green; Co^{++}, brown green; Ni^{++}, blue; Rh^{+++}, no colour; Pd^{++}, yellow brown; $IrCl_6''$, no colour; $PtCl_6''$, no colour

In conclusion it must be emphasised that the colours obtained are due to the complex ion and are entirely different from the colours observed with rubeanic acid or trisodium pentacyanoamminoferrate alone or with a mixed solution of rubeanic acid and potassium ferrocyanide.

The work described in this Note has been carried out as part of the programme of the Chemical Research Laboratory, and this paper is published by permission of the Director of the Laboratory.

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February 8th, 1956

THE SEMI-MICRO DETERMINATION OF SULPHUR IN CYSTINE AND METHIONINE

MANY methods are available for the determination of sulphur in organic compounds, but few of them are directly applicable to routine semi-micro analysis. The method proposed in this Note is believed to have a number of advantages that would make it suitable for such work. It consists in the oxidation of organic sulphur to sulphate by the method of Koszegi and Barcsay[1] and conversion of the sulphate so formed to hydrogen sulphide with the reduction mixture of Pepkowitz and Shirley.[2] Similar reductions have been used by Luke[3] and Archer.[4]

METHOD

REAGENTS—

Potassium dichromate—Analytical-reagent grade.

Phosphoric acid—Analytical-reagent grade.

Ammoniacal cadmium acetate solution—Dissolve 6·3 g of cadmium acetate in distilled water; add 25 ml of ammonia solution, sp.gr. 0·880, and make up to 250 ml with distilled water.

Reduction mixture—Mix together 100 ml of hydriodic acid, sp.gr. 1·7, 160 ml of concentrated hydrochloric acid and 30 ml of a 50 per cent. solution of hypophosphorous acid, and then boil the mixture for 20 minutes.[2]

PROCEDURE—

Take sufficient of the test material to contain 1 to 2 mg of sulphur and transfer it to a 25-ml round-bottomed flask with a B19 standard neck. Add 250 mg of potassium dichromate and 2 ml of phosphoric acid; fit a condenser and heat under reflux gently over a bunsen burner for 2 hours. When using a water condenser, it is advisable to tilt it at an angle to prevent the condensate from dropping directly into the boiling phosphoric acid. After 2 hours allow the system to cool and, when cold, add 10 ml of the reduction mixture. Fit delivery and exit tubes into the top of the condenser, and sweep air out of the apparatus with a stream of nitrogen. Boil the solution for 15 minutes, passing the exit gases through two boiling tubes each containing 10 ml of the cadmium acetate solution. The sulphur originally present in the sample is reduced to hydrogen sulphide and trapped in the cadmium acetate solution. Collect the cadmium sulphide on a 5·5-cm Whatman No. 1 filter-paper and wash it with 5 per cent. acetic acid; transfer the filter-paper and precipitate to 25 ml of 0·01 *N* iodine solution. Acidify, and then titrate the excess of iodine with 0·01 *N* sodium thiosulphate and from the results calculate the amount of sulphur present. Presumably any other suitable method for the determination of hydrogen sulphide, such as, perhaps, that of Archer[4] could be used.

A blank determination on the reagents should be carried out. When AnalaR chemicals were used, it amounted to 0·02 mg of sulphur.

RESULTS

Typical results for sulphur in various compounds are shown in Table I.

TABLE I

DETERMINATION OF SULPHUR

Compound	Weight taken, mg	Sulphur present, mg	Sulphur found, mg	Recovery, %
Cystine	4·46	1·19	1·19	100·0
Cystine	6·69	1·78	1·77	99·4
Cystine	8·15	2·17	2·17	100·0
Cystine	8·15	2·17	2·14	98·6
Methionine	6·79	1·46	1·48	101·3
Methionine	9·03	1·94	1·96	101·0
Methionine	9·03	1·94	1·92	99·0
Methionine	9·90	2·13	2·12	99·5
Methionine	9·90	2·13	2·14	100·5
Sulphanilic acid	10·97	2·02	2·02	100·0
Sulphanilic acid	10·97	2·02	1·98	98·0
Elementary sulphur	1·80	1·80	1·81	100·6

The very low solubility product of cadmium sulphide removes the need for solubility corrections, while the fact that there is no transference of the sample until the hydrogen sulphide is swept out in the gas stream minimises the possibility of manipulative errors.

For routine analysis it would seem to be possible to set up banks of flasks on the lines of the multiple Kjeldahl units. By this means it should be possible to carry out six or twelve determinations at once, if standard apparatus is used.

I express my thanks to the Directors of County Laboratories Ltd., for permission to publish this Note.

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March 29th, 1956

AN IMPROVED METHOD FOR THE QUANTITATIVE DETERMINATION OF AMINO ACIDS BY MEANS OF INDANETRIONE HYDRATE

The colour reaction between α-amino acids and indanetrione hydrate (triketohydrindene hydrate) has been studied extensively by several workers.[1,2,3,4,5,6,7,8] This method is very sensitive for qualitative determinations, but the colour yield is not reproducible unless the conditions of the reaction are controlled strictly. Several workers have reported that the colour yield per given quantity of amino acid decreased markedly with decrease in the concentration of the amino acid. Moore and Stein[9] observed the effect of dissolved oxygen and found that better results were obtained when the reaction occurred in tubes evacuated to a pressure of 20 mm of mercury. They prevented the oxidation side-reaction by using either stannous chloride[9] or hydrindantin.[10] More recently, work by Meyer and Riklis[11] has shown that certain cations present in the reaction mixture will cause a marked decrease in the speed of colour formation and will also change the absorption maximum of the coloured complex formed by the reaction between an α-amino acid and indanetrione hydrate.

Indanetrione hydrate obtained from commercial sources is known to vary in its colour yield with a standard quantity of a particular amino acid according to the quality of the batch of reagent. In this laboratory the analysis of amino-acid mixtures by a modification of the Moore and Stein method[9,10] has been improved by recrystallising the indanetrione hydrate. The use of freshly recrystallised indanetrione hydrate did not prevent an occasional reaction when the colour yield with a standard quantity of leucine was low. This difficulty has been overcome by treating a solution of the commercial sample of indanetrione hydrate in methyl Cellosolve with cation-exchange resin and omitting the recrystallisation procedure.

Method

Reagents—

Indanetrione hydrate stock solution, 4 per cent.—Convert a cation-exchange resin (Dowex 50 or Zeo-Karb 225) to the hydrogen form by putting it on a sintered-glass filter-funnel and treating it with 4 *N* hydrochloric acid. Wash the resin with distilled water until the pH of the filtrate is 5·5 and then wash it with methyl Cellosolve. Add this resin to a 4 per cent. solution of indanetrione hydrate in methyl Cellosolve. Store in a dark bottle under nitrogen.

Sodium acetate buffer solution, 4 N (pH 5·5)—Dissolve 544 g of crystalline sodium acetate, $CH_3COONa.3H_2O$, in water, add 100 ml of glacial acetic acid and dilute to 1 litre.

Stannous chloride, crystalline—Analytical-reagent grade.

Procedure—

Prepare a 2 per cent. indanetrione hydrate solution by mixing 50 ml of 4 per cent. stock solution with 25 ml of water and 25 ml of sodium acetate buffer solution (pH 5·5) and add 0·08 g of stannous chloride.

Mix a 2·0-ml solution of the amino acid in a sodium citrate buffer solution (this may vary from 0·2 *M* at a pH 3·15 to 2·0 *M* at pH 5·0) with an equal volume of 2 per cent. indanetrione hydrate reagent and heat the mixture in a water bath at 100° C for 30 minutes. Cool the reaction mixture, dilute it to 10 ml with aqueous ethanol (1 + 1) and determine the optical density by means of a Unicam SP350 spectrophotometer.

Dowex 50 (4 per cent. or 10 per cent. cross linkage) and Zeo-Karb 225 (water regain 1·55)

have been used with success. A layer of resin approximately ½ inch in depth was used to de-ionise 500 ml of the 4 per cent. indanetrione hydrate stock solution.

Results

The colour produced by the reaction of 0·4 micromoles of leucine in a 2·0-ml sample and 2·0 ml of the 2 per cent. indanetrione hydrate gave, when diluted to 10 ml, a consistent optical density of 0·76 ± 0·01 in the Unicam SP350 spectrophotometer. This improved method of purifying the indanetrione hydrate avoids the loss of 10 to 30 per cent. of the reagent that occurs on recrystallisation. The blanks, obtained by allowing 2·0 ml of the 2 per cent. indanetrione hydrate solution to react with 2·0 ml of 0·2 *M* sodium citrate buffer (pH 3·15) and diluting to 10 ml, had an optical density that varied between 0·04 and 0·06.

The following results are typical analyses of protein hydrolysates obtained after treatment of the respective protein with 6 *N* hydrochloric acid at 105° C for 24 hours—

Sample	Load, μg of N	Recovery, μg of N
Cryoglobulin hydrolysate 1	1017	1043
Cryoglobulin hydrolysate 2	763	776
Penicillinase 5B hydrolysate	1088	1070

The load (μg of N) indicates the total nitrogen (determined by micro-Kjeldahl technique) in the protein hydrolysate added to the column of Zeo-Karb 225 (water regain 1·55) resin used to separate the amino acids. The figure for recovery (μg of N) in the table indicates the summation of nitrogen contents of the individual amino acids and ammonia recovered in the eluates from the column of cation-exchange resin. The discrete peaks on the elution curve obtained by gradient elution from a column of Zeo-Karb 225 (water regain 1·55) resin permit the quantitative determination of the individual amino acids.

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S. Jacobs
January 23rd, 1956

Ministry of Agriculture, Fisheries and Food and Ministry of Health

STATUTORY INSTRUMENT*

1956—No. 919. The Milk and Dairies (Channel Islands and South Devon Milk) Regulations, 1956. Price 3d.

These regulations, which came into operation on July 1st, 1956, require that all milk for human consumption sold as Channel Islands, Jersey, Guernsey or South Devon milk shall contain not less than 4 per cent. by weight of milk fat.

* Obtainable from H.M. Stationery Office. Italics indicate changed wording.

British Standards Institution

NEW SPECIFICATIONS*

B.S. 332: 1956. Liquid Driers for Oil Paints. Price 3s.
B.S. 2713: 1956. 2-Ethoxyethanol (Ethylene Glycol Monoethyl Ether). Price 3s.
B.S. 2714: 1956. Methyl*cyclo*hexanol. Price 2s. 6d.

AMENDMENT SLIP*

A PRINTED slip bearing amendments to a British Standard has been issued by the Institution, as follows—

PD 2495—Amendment No. 2 (June, 1956) to B.S. 691:1953. Clinical Maximum Thermometers.

* Obtainable from the British Standards Institution, Sales Department, 2 Park Street, London, W.1.

Book Review

TRAITÉ DE MICRO-ANALYSE MINÉRALE: QUALITATIVE ET QUANTITATIVE. Volume II. By CLÉMENT DUVAL. Pp. 448. Paris: Presses Scientifiques Internationales. 1955. Price 3000 fr.

This second volume of Professor Duval's ambitious treatise comes fully up to the standard of the first (*Analyst*, 1955, **80**, 79) and it is not proposed to repeat here the comments about the ability with which the material has been collected and presented or the undoubted value of the series to all workers in the field of analytical chemistry.

The treatment of the individual elements follows the pattern used in the previous volume. Those elements now presented comprise the transition elements of Group IV (Ti–Th), Group V (V–Pa), Group VI (Cr–U together with the post-uranium elements up to Cf) and Group VII (Mn–Re). This volume will therefore be particularly welcome, since it deals with so many elements that have only really come to the fore relatively recently, elements that are of such rapidly increasing importance that it is essential to have available a reliable account of their analytical chemistry.

Those who already possess the first volume of the series will need no urging to add to it the present volume, and will await with impatience the remaining two volumes. CECIL L. WILSON

Publications Received

ESSAYS IN BIOCHEMISTRY. Edited by SAMUEL GRAFF. Pp. x + 345. New York: John Wiley & Sons, Inc.; London: Chapman & Hall Ltd. 1956. Price $6.50; 52s.
Twenty-five essays written in honour of Hans Thacher Clarke of Colombia University on his retirement.

FLOW OF GASES THROUGH POROUS MEDIA. By P. C. CARMAN, M.Sc., Ph.D. Pp. x + 182. London: Butterworths Scientific Publications; New York: Academic Press Inc. 1956. Price 30s.; $6.00.

Erratum

JULY (1956) ISSUE, p. 427, authors' names. In some copies of this issue the initials of G. W. C. Milner appear incorrectly as C. W. C.